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Abstracts of Scientific Papers

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Lectures and Oral Communications

PL-2 High Throughput and Traditional Phenotyping: How International Efforts Can Help Our Research

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We all do phenotyping, in our work, and in the rest of our lives. As technicians, veterinarians and scientists, we assess health and wellbeing of our animal patients and research subjects. We treat their disease conditions, based on observed or measured phenotypes from direct examination and tests. We analyze and report dysmorphologies or test results that deviate from the findings (phenotypes) in 'normal' or control animals. In academic research settings, most of our animal phenotyping for research may be considered to be Hypothesis Driven. In this context phenotyping (testing) aims to test specific hypotheses about specific disease conditions or biologic mechanisms. In industry or pharmacology settings, most of our animal phenotyping (testing) may be considered to be Purpose Driven, generating comprehensive datasets to determine if a specific compound is safe, effective, toxic, or carcinogenic. In high throughput phenotyping, as in the international mouse phenotyping consortium (IMPC), phenotyping (testing) may be considered to be Hypothesis Generating, also generating comprehensive datasets, but aiming for the data to be broadly relevant to multiple research areas, to improve the utility of mutant mouse tools for diverse hypothesis driven research. If your research involves a gene or molecular pathway, look it up. Relevant mutant mice, data, and protocols may offer new information and tools for testing new hypotheses. Phenotyping pipelines, protocols, data will be discussed, emphasizing their availability and utility to diverse research areas.

S01-1 FELASA Is Not Just a Congress Organizer!

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The Federation of European Laboratory Animal Science Associations, FELASA, is a truly pan-European scientific organization with 19 Laboratory Animal Science (LAS) Associations as members representing 26 countries and two LAS organizations with observer status. FELASA is most visible through the publication of guidelines and recommendations and through its triennial congresses. However, FELASA is most active as a stakeholder with the European Commission and the Council of Europe in Brussels representing the views and opinions of the European LAS community. Its mission is to advance and co-ordinate the development of all aspects of LAS and practice in Europe; to act as a focus for the exchange of information about LAS and to establish and maintain appropriates links with (inter)national and governmental bodies as well as other international organizations concerning topics on or related with LAS. FELASA is actively involved in the transposition of EU Directive 2010/63/EU into primary legislation and the drafting of guidance for secondary legislation. FELASA aims to improve and promote education and training in LAS and does so amongst others by its accreditation program. Believe it or not, but FELASA does all this through the dedication of a core of volunteers. As this core is never large enough, additional expertise, views, input and support are more than welcome. Therefore, don't hesitate to contact us and offer your active participation: www.felasa.eu; email: info@felasa.eu.

S01-2 FELASA Working Groups: Aims and Overview

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One important issue for FELASA is to establish and maintain working groups. These working groups should aim for guidelines, recommendations and reports. FELASA working groups are composed of international experts in the field of LAS and other areas of science relevant to the topic of the working group. The outcome of the working groups are available as full papers from FELASA's website and linked to peer reviewed articles. FELASA has now 7 active working groups. Two years ago a liaison between FELASA and AALAS was established. Two joint wg were identified, one on harm-benefit analysis of animal studies and one on health monitoring of rodents. The harm - benefit WG group addresses overarching ethical principles that need to be considered when engaging in animal research are driven by the same doctrines of replacement, reduction, and refinement in both United States and Europe. A jointly produced document would therefore be applicable in both geographical areas, and serve as an important guideline, that members of the scientific community could access when working towards harmonization of ethical reviews. The aim of the health monitoring WG is to find a way to harmonize between USA and Europe. This is important as collaboration between our continents is increasing. Transfer of rodents between institutions occurs continuously. The logistics involved in these transfers are becoming more and more complex due to the scientific value of the animals, the legal hurdles that need to be addressed for inter-nation/country shipments, and the possibility of transmitting unwanted microbiological agents between institutions.

S01-3 FELASA Accreditation of Health Monitoring Programs and Testing Laboratories Involved in Health Monitoring

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FELASA issued guidelines in 2010, which define the operation of a FELASA board responsible for the accreditation of health monitoring programs and for testing laboratories. This board evaluates laboratories and programs after voluntary application for accreditation. Official FELASA accreditation can be given to health monitoring schemes and/or to laboratories if they conform to the quality standards described in the FELASA recommendations. FELASA hopes that this accreditation program will increase the significance and reliability of health monitoring reports and promote further standardisation of laboratory animals. Applicants should contact the secretariat through the FELASA

Internet sites and will receive the application package and additional information. Applications should be submitted electronically. Each application is thoroughly examined and discussed within the accreditation board, which will usually decide upon the application, perhaps after additional information has been obtained from the applicant. Site visits may exceptionally be conducted. Until now several commercial health monitoring laboratories from different European countries, but also from the USA and Australia, expressed their interest in FELASA accreditation; fewer European institutions including a commercial breeder asked for accreditation of their health monitoring programs.

S01-4 FELASA Education and Training Accreditation System

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For several years FELASA has been developing guidelines for the education and training of all categories of personnel working with experimental animals. More recently, in 2003, FELASA established an accreditation system for teaching programs. This quality assurance system is intended to assist in the development of high quality educational programs for all laboratory animal categories of personnel. Currently more than 40 courses run in most EU countries have been accredited, or are in the process of being accredited, by FELASA. There is also increasing interest from outside Europe to adopt both FELASA guidelines for training and to obtain FELASA accreditation. At present the FELASA Accreditation board for Education and Training is revising its accreditation scheme to adapt it to the new challenges resulting from the implementation of Directive 2010/63EU. By providing quality assessment of all the components of the LAS training process, our aim continues to be to make it possible for all European countries to offer harmonized, high quality education and training programs in laboratory animal science; to increase quality standards and to facilitate the interchange of personnel across Europe. During the presentation, the update of the accreditation system will be presented and discussed. All FELASA guidelines and information on the accreditation system can be found at http://www.felasa.eu

S02-1 A Framework for Education and Training in Europe: Overview of the Work of the Expert Working Group

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In 2012, the Commission established an Expert Working Group to develop a common education and training framework for the EU to fulfill the requirements under Articles 23 and 24 of Directive 2010/63/EU. The common framework aims to assure the competence of all persons involved in the use, care and breeding of animals for scientific procedures, and to facilitate free movement of personnel. The new Directive shifts the focus from educational/training background to the demonstration and maintenance of individual competence. To respond to these new requirements, the framework establishes a flexible, output-based (Learning Outcome) modular training structure together with principles and criteria for supervision, competence assessment and continued professional development. Agreement at EU level on general principles will assist those developing training courses to work towards common, acceptable standards. This in return should result in a wider offering of available training courses to promote the aims of availability, accessibility and affordability. Principles for a mutual approval/accreditation framework are equally required as the basis for mutual acceptance of training carried out elsewhere. A system to promote mutual recognition and quality of training at an EU level should be cost effective with minimal administrative burden. Education and training is the competence of Member States, therefore each Member State decides how this general guidance is to be implemented. Nevertheless, Member States have provided their continuous support at all stages of the work. The work will be completed in July 2013.

S02-3 Accreditation and Mutual Acceptance of Laboratory Animal Science Courses within Europe

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Directive 2010/63EU provides an excellent opportunity to harmonize education and training in LAS in Europe. The European Commission (EC), by means of expert working groups, is setting up the basis for a common E&T framework that will ensure competence of personnel and facilitate the mutual acceptance across Europe. However, it is necessary now to develop a system for approval/accreditation for training modules/courses to assure confidence in the quality of the training and their assessment. FELASA has been playing a very important role accrediting courses across Europe and it is willing to play a leading role in the development of the process that the EC proposes. The principles for the approval/accreditation process must be: independence, consistent standards, built of confidence and trust and proportionate and affordable. During the next months it will be necessary to develop the process in an effort where all partners need to work together and where training providers and those with experience in evaluating training programs need to be involved. The EC is proposing an EU Platform and Information Portal on Education and Training where accrediting bodies, course providers, Member States and National Contact Points will be represented. Some of the work ahead is: 1) to define criteria for Accrediting /Approval Bodies; 2) to recognize and maintain a list of Accrediting /Approval Bodies and courses; 3) to define criteria for minimum quality standards; 4) to provide advice and information to all parts and particularly to course providers and Accrediting/Approval bodies; 5) to sort any other issues that might impede mutual acceptance.

S02-4 Selecting and Training Members of Animal Welfare Bodies

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Directive 210/63/EU requires all establishments that breed, or use animals in scientific procedures to set up an Animal Welfare Body (AWB). The AWB is responsible for advising on "… the welfare of animals in relation to their acquisition, accommodation, care and use". It must also advise on the 3Rs, establish and review internal operating processes in relation to the welfare of animals, follow the development and outcome of projects and advise on rehoming schemes. To discharge these responsibilities effectively, the AWB will need its members to have wide ranging knowledge and considerable experience in the issues it has to consider, together with the kind of personal qualities that ensure constructive communication. Training requirements for the four categories of personnel (a-d) under the Directive are being developed at the time of writing, but training for AWB members has not yet been discussed. This presentation will review any selection and training requirements for committees with similar roles - for example, institutional ethics or animal care and use committees - as a starting point to stimulate discussion of how AWB members may be selected and what kind of training they may need.

S03-1 Why Were Cephalopods Included in Directive 2010/63/EU?

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The inclusion of an entire class of invertebrate animals-the Cephalopoda (phylum Mollusca) -in EU Directive 2010/63 marks a major

change in EU wide animal legislation and in principle gives the same protection to "all live cephalopods" as has been afforded to vertebrates. In the EU species of octopus, cuttlefish, squid and nautilus are variously used in research on behavior, neuroscience, physiology, genomics and aquaculture. A key driver for the recommendation by the EFSA Panel on Animal Health and Welfare (2005) was their assessment of the evidence for the experience of pain. Whilst inclusion of cephalopods in the Directive is appropriate, particularly adopting the precautionary principle, there is a relative paucity of evidence for pain perception in comparison to vertebrates. The following aspects will be reviewed; presence of nociceptors; brain complexity and sensory pathways; anesthesia and analgesia; behavioral and learned responses to noxious stimuli. Knowledge gaps requiring research to inform the development of Guidelines for the Care and Welfare of Cephalopods (www.CephRes.org), and particularly objective identification of signs of pain and suffering be discussed.

S03-2 The Development of Consensus Guidelines for Care and Welfare of Cephalopods: Key Recommendations

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From January 2013, scientific projects involving cephalopods are regulated by Directive 2010/63/EU. This is the first time for an entire class of invertebrate species, and a challenge for the scientific community, care takers, veterinarians, laboratory animal scientists, organizations and regulators. The inclusion of all living cephalopods (larval and adult forms) in the Directive has a number of practical implications, including: supply of animals, transport, housing and handling, anesthesia, criteria for recognizing pain, suffering and distress, application of humane end-points, and euthanasia. Such implications have been only marginally explored till now. We will present a review of the research effort dedicated to this topic, how the challenge has been approached by the 'cephalopod-community', in a coordinated effort (co-chaired by FELASA, the Body Group, UK, and CephRes-ONLUS). This venture will generate within 2013 the first edition of "Guidelines for the care and welfare of Cephalopods in research". I will overview the process, the level of consensus achieved, and the major results of this endeavor. The characteristics of cephalopod species such as richness in the behavioral repertoire, diversity of the adaptations to the marine realm is a challenge that we are only just approaching systematically.

S03-3 Challenges in Applying Guidelines for Care and Welfare of Cephalopods to Daily Practice: A View from a Researcher and Caretaker

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Researchers have often compared cephalopods to vertebrates, showing that their cognitive skills, as well as neurobiological and behavioral complexity rivals, and in some cases even surpasses, that of vertebrates. Unfortunately, one of the first reactions to the inclusion of cephalopods in the Directive was skepticism; claiming that the Directive would impede, and perhaps even put an end, to all research on cephalopods (Nature News 12/4/2011). Indeed the new situation gives rise to several practical and conceptual problems for researchers, regulators, veterinaries and laboratory animal technologist much more to contribute to the scientific world, from basic science to bio inspired robotics. Now, we as cephalopod researchers must use the new Directive as a guideline, not a hindrance, for our work. As a response, a new opportunity has arisen in the creation of a collaboration of cephalopod researchers in a platform called CephRes-ONLUS dedicated to the promotion and facilitation of cephalopod research in Europe. In my presentation I will focus on the changes to be expected for those working with cephalopods due to the Directive 2010/63/EU, from day to day routines to experimental protocols. In addition I will give specific examples of experiments conducted today in the field of cephalopod research and how these will be influenced by the new regulations. European Directive gets its tentacles into octopus research.

S04-1 The AALAS-FELASA Liaison Body

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The AALAS-FELASA Liaison Body was founded in 2011 with the aim of providing a mechanism for developing activities that support the global advancement of responsible use of animals in support of scientific advancements. The Liaison Body considered that, although country-specific legal frameworks may impact on specific operational standards, professionals within the organizations share the same overarching principles relative to responsible animal care and use. The Liaison Body is composed of three elected leaders from each organization .One of the key methods for achieving the goals set forth by the Body has been the establishment of AALAS-FELASA joint working groups. These groups focus on researching and producing recommendations on topics of common interest that are lacking in guidance/definition. The first two joint working groups have been already established and are working on two important topics: Health Monitoring of Rodents for Animal Transfer; and Harm-Benefit Analysis for Animal Studies. The Liaison Body has already hosted two scientific sessions at the AALAS Meetings in 2011 and 2012. The first one introduced the Body, compared the US and European legal frameworks, and discussed initial ideas for the health monitoring of rodents for animal transfer. In 2012, the progress of this same working group was presented. The FELASA Congress 2013 is the third opportunity to present the progress of the working group on health monitoring of rodents for animal transfer, as well as the working group on harm-benefit analysis for animal studies. The next presentations will describe the status of both working groups.

S04-2 Update from the AALAS-FELASA Working Group on Health Monitoring of Rodents for Animal Transfer

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Transfer of rodents between institutions for research purposes occurs continuously between animal facilities in the USA, Europe and other parts of the world. The logistics involved in these transfers is complex due to the scientific value of the animals, the legal hurdles that need to be addressed for international shipments, and the risk of transmitting unwanted microbiological agents between institutions. Receiving institutions usually require information on the health status of the animals from the institution of origin. This information can be very variable and difficult to interpret, which very often leads to costly and time-consuming efforts that may or may not be necessary before animals are introduced into the recipient's colony. A consensus on minimum health monitoring recommendations and presentation format, which can be applied for international transfer of rodents is anticipated to protect the welfare of animals and facilitate the transfer process thereby saving on resources. The working group hosted a workshop during AALAS-2012 and will present at FELASA-2013. Topics are: major differences between current 'health information' shared between European and USA based institutions; Mustrac - a software tool developed to speed up the process of animal transfer between research institutes in the USA; the results of an internet poll on health monitoring and shipping with over 900 respondents and the first draft of a global health report format for discussion.

S05-1 Implementation of the New European Directive 2010/63 in Baltics

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New European Directive 2010/63, accepted by European Parliament on the 22th of September of 2010 introduces new period in laboratory animal science. Implementation of the new Directive had to be done till the first of January 2013, therefore it is high time to review how this process underwent and what are results. Three Baltic countries - Lithuania, Latvia and Estonia - are very closely located and have common history, therefore the main changes caused by implementation of new Directive - legal basis regarding use of laboratory animals, management of laboratory animal facilities, authorization of experiments based on the use of laboratory animals, classification of pain, statistics of laboratory animals used for scientific purposes, legal basis and functioning of Ethics committees - will be reviewed in one presentation.

S05-2 From National Legislation to the New European Directive 2010/63 in Republic of Bulgaria

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New scientific knowledge is available in respect of factors influencing animal welfare as well as the capacity of animals to sense and express pain, suffering, distress and lasting harm. It is therefore necessary to improve the welfare of animals used in scientific procedures by raising the minimum standards for their protection in line with the latest scientific developments. It is a huge challenge for Republic of Bulgaria as a new Member State to implement a measures in order to replace the use of live animals in procedures by other methods not entailing the use of live animals, the use of live animals continues to be necessary to protect human and animal health and the environment. This Directive is transposed into the national legislation and represents an important step towards achieving the final goal of full replacement of procedures on live animals for scientific and educational purposes as soon as it is scientifically possible to do so.

S05-4 Implementation of the Directive 2010/63 to the Polish Law

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Directive 2010/63 was adopted by the European Parliament and the Council in October 2010. All Member States, including Poland, should implement the Directive into their legislation and the relevant legal acts should be applied from 1st January, 2013.The Polish Law, enacted in 2005, regarding to using of animals in experiments is consistent in main points with the principles of the Directive. However, the newly enacting Law with detailed regulations and administrative provisions will be essential for a proper implementation of the Directive. Main points of the new Law concerning implementation of the Directive were drawn up by the team of experts in a middle of 2012 and passed to the further processing in the Polish Ministry of Science and Higher Education. The main assumptions of the proposed Law will be published and consulted publicly (for two weeks). Then the draft of the proposed Law will be written and open for public debate (for a month). Later the Project of the Law will be submitted to the Polish Parliament.

S06-1 Examination Strategies in Laboratory Animal Science

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The FELASA guidelines for education have been a tremendous success in the sense that the number of animal experimenters passing through these courses over the last two decades has increased dramatically. Also, scientists of today are in general far better trained in relation to various items of the category C curriculum and at many universities laboratory animal science courses have been integrated into the curriculums of master and PhD programs. To develop a successful career as scientist it is necessary to be critical and reflecting rather than to possess a factual knowledge on the time of assessment. As for any other type of academic course, laboratory animal science courses should therefore, develop candidates who can work critically with problems to find solutions; assessments used during such courses should reflect this. We have in our various laboratory animal science programs on category B, C and D level experimented with different types of examinations: Traditional oral, oral-practical, oral portfolio exams, combined practical-oral, traditional written, portfolio written, as well as online written exams. In general, we allow all helping tools. Each of these exam strategies has graded students over the entire performance scale, but emphasize different capabilities of the candidates. Our experience with these examination formats will be presented.

S06-2 Engaging with Students: Obtaining and Using Feedback to Enhance Quality

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Since our first FELASA Cat-C course in 2005, we have implemented various approaches to get constructive feedback and implement effective alterations. For systematic quanti-qualitative evaluation of the course components and to identify suggestions, problems or difficulties, we use standard student feedback forms distributed early in the course and returned during the last day. The most significant change in response to student feedback was introducing e-learning to increase schedule flexibility and optimize use of teacher contact time. This was made gradually with feedback from the students used to accompany the transition, giving us confidence to complete the transition of introductory theoretical content to e-learning and alerting us to necessary revisions to the e-learning platform. After the course, all student feedback is analyzed and systematically presented in a final report, discussed in a meeting with course organizers (and when possible teachers). At this point a course assessment is done, and alterations decided to improve future course editions. To engage with the students during the course, we have introduced two tutorial sessions where the students in working groups meet with the course organizers, around days 2 and 8 of the course. These sessions serve for the teachers to understand whether group composition and dynamics work appropriately and for the students to clarify doubts and ask specific questions. In order to provide methodologically solid evidence on our use of e-learning, we are presently validating an anonymous and quantitative questionnaire completed under standard conditions during the last course day.

S06-3 A Simple Modular Scheme of Education in Animal Experimentation: The Swiss Example

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Since 1999, education & continuing education in animal experimentation are mandatory to any person who will work with animals. Six roles are defined by Swiss law: Animal Caretaker (EU Function C&D/FELASA Category A1); Experimenter (EU Function A&D/FELASA Category B); Study Director (EU Function B&D/FELASA Category C); Resource Manager (No EU nor FELASA equivalent); Head of the Animal house (No EU equivalent/FELASA Category A2) and Member of the Ethical Board (No EU nor FELASA equivalent). Except the

Resource Manager and the Ethical Board member (who attends to only one day introductory course followed by continuing education), every other role has to attend an education course followed by continuing education. To achieve the Study Director education level, a simple modular approach has been implemented in Switzerland. He or she has to attend the Experimenter Education course, to practice during 3 years followed by another 40-hour course to be fully accredited by the Veterinary authorities. We will give an overview of the Swiss system of Education by discussing the content and timetable. The process of accreditation (two-tier) and auditing of courses will be described. Continuing training plays a role in the modular approach to education and will be briefly mentioned. Issues will be discussed, particularly on the use of live animals. With the high mobility of students and post-doc in life sciences, authorities are confronted to accreditation of scientists and technicians coming to work in Switzerland. A few examples of schemes of accreditation to fit Swiss legal requirements are presented.

S07-2 New Approaches to Pain Assessment in Laboratory Animals

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Pain in animals is of considerable public concern, particularly in those used in biomedical research. Pain compromises not only animal welfare, but also the validity of scientific results. In order to alleviate pain, we need to be able to assess its severity and duration effectively. Considerable advances have been made in assessing pain in animals through the evaluation of behavioral and postural changes. Behavior-based schemes have been developed for a range of species. They are considered more effective than those of the more subjective assessments of appearance and demeanor (that is, clinical signs) and offer a more immediate cage/pen side assessment of pain than objective measures of food/water intake, bodyweight change etc. However, behavior-based measures also have limitations including: being time consuming to develop and carry out, only offering an 'direct' measure of an animals' physical reaction to pain rather than how it makes them 'feel', and often being subtle and difficult to detect. The assessment of facial expressions exhibited in response to pain may offer a solution to these limitations. Facial expressions are used in clinical assessments of pain in humans, particularly in those who are unable to communicate verbally. There is now an increasing body of literature demonstrating that facial expressions change in response to painful procedures in rodents and rabbits. If these facial expressions are a direct measure of pain, then this may offer a new method of assessing pain in animals.

S07-3 Chronic Pain Assessment

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Long-term pain is a major challenge to clinical practice and basic science, and causes a much greater impairment in the patient's quality of life than other pain syndromes. Pain is not only a result of an afferent noxious input but is also dependent on changes taking place in the nociceptive transmission system. Chronically painful conditions may be associated with central nervous system sensitization (CS) and result in altered sensory processing and facilitated nociceptive transmission (spontaneous pain, hyperalgesia and allodynia). Chronic pain can be subdivided into four major categories, namely neuropathic pain, nociceptive pain, mixed pain and idiopathic pain. These conditions are generally difficult to diagnose and even harder to treat. The availability of quantitative measures of chronic pain that are valid and reliable in clinical patients is crucial for the development and testing of interventions designed to decrease such pain. Quantitative Sensory Testing (QST) assesses somatosensory function by recording responses to external stimuli of controlled intensity (for example, thermal, mechanical, electrical, vibration) and is becoming a useful tool to provide insight into the complex pathophysiology of the chronic pain categories, testing for secondary hyperalgesia and CS.A number of animal models of chronic pain have been developed over the last decades. These models have provided valuable information about QST alterations and the neurobiological signature of chronic pain, however this information is lacking in other laboratory animals or humans with naturally occurring painful conditions. Furthermore, chronic pain may impact or bias research results in many fields.

S07-4 Sustained Release Analgesia in Laboratory Animals

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Provision of post-operative analgesia to laboratory animals is not only an ethical imperative, but also an important refinement that can improve research outcomes. The choice of analgesics commonly selected varies little, even across species. Opioids such as buprenorphine and butorphanol, and non-steroidal anti-inflammatories such as carprofen, ketoprofen, and paracetamol (acetaminophen) remain the most widely used drugs. The duration of action of these drugs varies from short-acting opioids to longer acting NSAIDS, but in general, in order to maintain therapeutic levels over a sustained period of time, most of these drugs require administration 1-3 times daily. Frequent dosing requires more personnel effort as well as more handling and potential stress to the animals. It also is likely to result in large fluctuations in blood levels over the desired treatment period. Analgesic formulations that provide a more sustained and steady state delivery offer potential to overcome these shortcomings in post-operative pain management. There are several newer preparations that have been developed either for human or animal use. This presentation will discuss options currently available as well as those in development for both rodents and larger animal species, including transdermal, depot, and oral formulations. Benefits and limitations of the different formulations will also be presented.

S08-1 International Council for Laboratory Animal Science (ICLAS): A Global Umbrella Serving All. Laboratory Animal Quality Programs

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The International Council for Laboratory Animal Science (ICLAS) was established as a global organization to promote and coordinate Laboratory Animal Science throughout the world particularly in developing countries. Two important priorities have been the promotion of education and training of personnel involved in work with animals and the promotion of harmonization of guidelines and regulations in the care and use of laboratory animals at a global level. Although ICLAS is very well known for harmonization and for the promotion of education and training, ICLAS also plays an important role in promoting quality definition and monitoring of Laboratory Animals used in research. The ICLAS Network for the promotion of laboratory animal quality (LAQN) has developed two programs: 1) the Performance Evaluation Program (PEP) for Diagnostic Laboratories that offers highly defined sera and/or microbiological samples to enable participating laboratories from around the world to self-assess their diagnostic performance; and 2) the Genetic monitoring program that aims to publish guidelines to provide guidance in relation to genetic monitoring of laboratory animals. At the session details of these programs and how to participate will be given.

S08-2 ICLAS Ethics and Animal Welfare Committee: Ethical Guidelines for Researchers, Editors, and Reviewers

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Many countries throughout the world have ethical guidelines for performing animal experiments. These guidelines differ according to cultural, historical and religious attitudes. Some countries even have no guidelines at all. Thus the goal of the ICLAS Ethics and Animal Welfare Committee is to set up basic ethical guidelines, which can be accepted by all member countries of ICLAS. Such general guidelines for researchers, editors and reviewers will be presented and discussed.

S08-3 ICLAS European Training Fellowship Program

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The European Regional Committee of the International Council for Laboratory Animal Science (ICLAS) has opened three training fellowship awards in laboratory animal science. This award is available to individuals from European countries/areas in the process of developing modern standards of animal care and use. The objective of the fellowships is to acquire new knowledge and/or technical competences in the care and use of laboratory animals, that can be used later in the applicant´s countries. This fellowship can be achieved in one of the host institutions proposed by ICLAS or in an institution proposed by the applicant. Fellowships can be awarded at three levels: Technician level; Animal facility manager/animal welfare officer/veterinarian level; and Animal user scientist level. The program is open to individuals with a career goal of taking care of animals, or managing an animal research or production facility program in their country, or performing animal research. Preference is given to individuals who otherwise would not have funding for international travel to and experience in activities of this kind. This ICLAS initiative, sponsored by several commercial companies, is trying to help improving laboratory animal science and welfare in those European areas where it is more needed. Information on the first awards and the process followed so far will be offered.

S08-4 How ICLAS Can Help in Europe: The Turkish Example

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International Council for Laboratory Animal Science (ICLAS) has been contributing to ethical use and care of laboratory animals in research worldwide. Promoting regional scientific activities and collaborating with national associations through regional committees is extremely important for ICLAS to promote laboratory science. One of the important tasks for ICLAS has been to encourage association to organize regional scientific meetings. This is highly important especially for relatively inexperienced national associations to receive international help. In case of the Turkish LASA, collaboration with ICLAS has made a significant impact by means of national and international recognition of the Turkish LASA and our effort to promote ethical use and care of lab animals as well as quality of animal experiments. Although there had been individual effort for international collaboration, the Turkish LASA was legally established in 2009, but made a significant progress as far as scientific activities and collaboration with government authorities. We believe affiliation to ICLAS is one of the reasons behind this success story. Importantly, ICLAS encouraged the Turkish LASA to organize the 3rd East Mediterranean ICLAS Regional Symposium in June 2011 in Istanbul. Around 300 participants form over 30 different countries participated in the symposium. A number of Turkish lab animal scientists as well as people from national authorities had a chance to interact with international colleagues. Thus, ICLAS regional scientific activities are critically important to reach out lab animal people and synchronize/harmonize their efforts.

S09-1 Genetically Engineered Pig Models for Diabetes Research

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Recent progress in genetic engineering facilitated the generation of tailored pig models for diabetes research. Transgenic pigs expressing a dominant negative receptor for the incretin hormone glucose-dependent insulinotropic polypeptide (GIP) demonstrated a crucial role of the GIP system for the physiologic age-related expansion of pancreatic beta-cell mass. Moreover this animal model shares important characteristics of type 2 diabetes mellitus: impaired incretin effect, reduced glucose tolerance and insulin secretion, and a progressive reduction of beta-cell mass. More recently we used this model to search for metabolic biomarkers which are associated with progression in the pre-diabetic period and identified specific amino acid and lipid signatures as candidate biomarkers. Further, we created the first pig model for permanent neonatal diabetes by expression C94Y mutant insulin in the beta-cells of transgenic pigs. In addition to their use as biomedical models, pigs may also serve as organ and tissue donors for xenotransplantation. Transplantation of encapsulated porcine pancreatic islets to type 1 diabetic patients with severe unaware hypoglycemia has already entered clinical studies, but encapsulation may shorten the lifespan of the islets. Therefore, in order to overcome the rejection of pig islets by human T cells, we generated transgenic pigs expressing the optimized CTLA-4Ig variant LEA29Y in the pancreatic beta-cells. Islets from LEA29Y transgenic pigs rescued diabetes and were protected against rejection in a humanized mouse model.

S09-3 PET Imaging in Göttingen Minipig Models of Neurologic and Neuropsychiatric Disorders and Therapeutic Interventions

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Progress in neuroscience research often involves animals since no adequate alternatives exist to animal models of living systems. The search for the most appropriate animal species for a given study is often driven by multiple considerations such as time, size and cost. In brain imaging studies such as positron emission tomography, the size of the brain and the feasibility to image specific structures become important issues. In recent years, the pig has become a model of interest for neuroscience studies. Pigs have a gyrencephalic brain of a size similar to primates, allowing the identification of cortical and subcortical structures by imaging techniques. Furthermore, research in pigs provides the opportunity for transgenic manipulations with less concern for availability, cost and handling restrictions. The major issue for longitudinal studies in pigs is the rapid growth precluding chronic studies. To circumvent this issue, easily trainable strains like the Göttingen minipig have been specifically developed for research and long-term studies. We will discuss recent data from longitudinal *in vivo* brain imaging studies in models of Parkinson disease and sugar addiction as well as the mechanisms of antidepressant brain stimulation therapies, electroconvulsive therapy and vagal nerve stimulation, in Göttingen minipig with a special focus on monoaminergic neurotransmission.

S09-4 Validating the Cognitive Holeboard Task for Pigs

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The majority of translational studies use rodents as model animals for studying behavioral (dys)functions and the effects of putative therapeutics. However, larger animals such as pigs have recently received increasing attention as model animal. Pigs are highly social and intelligent and can be trained to perform complex cognitive tasks. Moreover, they have relatively large brains and are physiologically more similar to humans than rodents. The use of pigs in research has a number of advantages: in particular, they are more readily available and are raised under more controlled conditions than, for example, primates and companion animals. These animal models are expected to close the gap between research in rodents and humans. Cognitive tasks adapted from rodent and primate research have been adjusted to suit the physical and cognitive abilities of pigs. One of these tasks assesses spatial learning in the cognitive holeboard, a free choice maze in which rewards can be found in a subset of a number of spatial locations. In a recent study, sows were treated chronically during the last trimester of pregnancy with a xanthine oxidase inhibitor. This compound is already clinically applied to acutely treat human neonates suffering from asphyxia. Our study compared cognitive performance of low and normal birth weight piglets from treated and untreated sows in the holeboard task. No differences in learning were found. As no undesirable side effects were observed, prolonged prenatal treatment with this xanthine oxidase inhibitor can be regarded as safe. Efficacy of prenatal treatment still needs to be confirmed.

S10-1 Breeding Colony and Pathology Problems

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NHP breeding centers in Europe have greatly evolved over the past decade. In particular, the European network Euprim Net has allowed coordinating some various aspects of the breeding process. In our presentation we will summarize the various sanitary problems (infectious and noninfectious) that may need to be faced both inside the breeding centers, and inside the research center NHP facilities. Then, we will see which new measures and recommendations can be taken in order to improve the sanitary surveillance of the breeding centers.

S10-2 NHPs Quarantine and Health Screening

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Importation of nonhuman primates (NHPs) in European Union (EU) for experimental and other scientific purposes leads to an average annual flow of 10 000 animals entering in European countries from different sources. Self – sustaining colonies in EU are indeed not sufficient to fulfill research needs, and lots of NHPs used in procedures are bred in a non-European country (Mauritius, China, Vietnam, Philippines, Barbados, etc.). This means high diversity in origins and genetic strains but also in sanitary status. NHPs shipped to EU must be quarantined and monitored for evidence of infectious diseases transmissible to humans. National and international laws and recommendations define the NHPs health status requirements and import procedures. But no homogeneous regulation is defined for quarantine in EU, which leads some Institutes using NHPs to take the initiative and responsibility of health screening programs once animals arrive in their facilities. We will review health data in quarantine of NHPs from different sources (mostly *Macaca* spp.) over the past years by focusing on main sanitary issues. Prevalence of tuberculosis, herpes B virus, retrovirus, *Shigella*, *Salmonella*, and *Yersinia*, appear to lessen in statistics. Quarantine procedures, including 4 to 6 weeks of isolation, consecutive tuberculin skin tests, serology, fecal culture, parasitology exams, and baseline blood values will be discussed. Thanks to efforts of breeders, new methodology, regular health screenings of the animals and practice of quarantine when arriving in EU, NHPs health status has improved for the last years and ensures the quality of animals entering research programs.

S10-3 Macaque Breeding Management: Introduction, Success Rate and Tenure Length of Alpha Males

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With the implementation of the new European Directive 63/2010 even more effort is put into improving primate welfare. In order to be able to offer the best experimental animals for biomedical research, the BPRC maintains a well-kept, self-sustaining breeding colony of rhesus macaques. Over 800 animals live in large social groups that mimic natural grouping patterns. This implies that groups consist of 1-4 matrilines, that animals are not taken out of their natal group before being subadult and that a new, unfamiliar adult male is introduced every 4-5 years. Introduction of new males in this despotic species can cause severe trauma and needs specific introduction protocols. We will show data from more than 50 introductions over the previous 15 years. The success rate of males of different breeding status and the trade-off between produced number of offspring and introduction risk will be discussed: is there an optimal tenure length? Although the introduction of unfamiliar adult males in a large group is always risky we feel that our procedures will lead to "better" experimental animals. Our data show that BPRC's unique breeding strategy does not have a negative effect on interbirth intervals of females, and that it is not necessary to wean babies at an early age and raise them in peer groups. We demonstrate that it is possible to keep and breed rhesus macaques for research purposes in groups that fully meet their social, behavioral and environmental needs.

S10-4 Withdrawn

S10-5 Defining, Observing and Assessing Abnormal Behavior in Nonhuman Primates

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The extant behaviors exhibited by a primate are often the initial and primary indicator used to gauge the animal's welfare. Therefore, it is important that personnel working with primates are adequately trained to recognize the behaviors (normal and abnormal) expressed by the animals and understand their significance vis-a-vis the animals' overall wellbeing. In addition, staff should be aware of the manner in which their own actions and behavior may impact the primates. Optimally, the animals will exhibit a broad range of species-typical behavior. However, some abnormal behaviors may develop due to inappropriate early rearing conditions and may be very challenging to mitigate; others may be a reflection of an impoverished housing environment and thus may be addressed more readily. Further, some kinds of species-typical behavior may be better indicators of good welfare than others. For example, reproductive success is often considered to be a positive indicator of good welfare, but not all captive primates are of breeding age, and some might be deliberately maintained in nonbreeding situations; also, some types of reproductive failure are not necessarily related to poor welfare. Conversely, some abnormal behaviors may be more important indicators of compromised welfare than others, and rankings of the welfare consequences of different types of abnormal behavior have been developed. Thus, the assessment of the primates' welfare should rely on carefully selected, meaningful metrics. In addition, the assessment should consider the effect of the observer, the individual traits of the animal, and the context in which the animal observation is made.

S11-1 Revision of FELASA Guidelines for Health Monitoring of Rodents and Rabbits

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Health monitoring (HM) is the cross-road where animal breeding, scientific experiments, biologic reagents and laboratory diagnostics meet. HM is a complex field that requires supervision by competent specialists. Success will depend on careful planning and correct implementation at all practical levels including selection of relevant animals, infectious agents, and laboratory methods, quality control, and interpreting and communicating test results. The guidelines for health monitoring of laboratory rodents and rabbits have been revised by the FELASA Working Group with additional input of the FELASA Board of Management, member-associations and other relevant professional organizations. The guidelines seek harmonization of HM programs, but emphasize at the same time that the diversity of contemporary animal colonies requires flexibility, because HM programs must adapt to local conditions and regulations. The revised HM guidelines describe both general and specific aspects of HM, including design of HM program, concept of microbiological unit, choice of agents and frequency of testing, selection of animals/tissues, test methods, interpretation of results, laboratory reporting and health reporting. A suggested list of agents for which to regularly monitor and organisms with the recommended frequency of monitoring for each animal species considered. Immunodeficient animals and biologic reagents also are discussed. New developments have received special attention. The increased use of cage-level containment where agents may occur at low prevalence difficult to detect is challenging. Another challenge in HM is that sentinels are increasingly being used to detect multiple contaminants, but there is no universally susceptible sentinel that is equally apt for all pathogens and test systems.

S11-2 Rodent Health Monitoring: Critical Steps with Regard to Technical Development and Innovation

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Within the last 100 years, health monitoring of laboratory rodents has been constantly adapted to new technical developments and diagnostic procedures. In principle, health monitoring programs are characterized by sampling strategies and frequencies, selection of agents and test procedures, as well as reporting. Common standards are defined, for example, by FELASA. As there is no obligation to follow current standards, all components of health monitoring programs might show great variation, often hindering comparison of hygienic situations in animal facilities. However, adaptation to given situations is necessary. For example, sampling strategies for health monitoring have been established in the past, but with development of IVC maintenance, determination of sampling sizes, test material and sentinel strategies have to be reviewed. In addition, various test procedures are currently available and need to be considered in health monitoring. Several classic microbiological and parasitological methods are still important procedures used in diagnostic laboratories. In addition, molecular methods are indispensable in our days and increasingly used even for replacement of traditional methods. Facility managers and laboratory animal veterinarians should be aware of the possibilities and limitations of the several methods in use as well as critical aspects of health monitoring that come with modern maintenance systems. Besides, they have to be aware of the increasing importance of microbial factors that are not currently standardized by hygienic monitoring.

S11-3 Health and Welfare in Genetically Altered Rodent Models: Developing Mouse Welfare Assessments

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Transposition of the European directive 2010/63/EU into law brings with it a greater emphasis on the assessment animal welfare and severity in medical research. For GA rodents this must take into account the mutations applied to the model as well as their general health and welfare. Developing assessments requires an understanding of the animal's standard behaviors and training for care staff and scientists alike to recognize deviations away from this normal status. Production and archiving centers make model access easier, but dependent on the information that travels with the animal, welfare issues may not be clearly conveyed. Factoring environment, consumables and husbandry changes that greet the animals mean potential unexpected welfare issues may manifest themselves. Therefore from a 3R's perspective on-going welfare assessments ensure the optimal condition of the animals and help define any potential detrimental effects that the growing colony may be exposed to. As with all animal facilities our institute recognizes the advantages of developing controlled welfare assessments on the colonies we manage. By understanding the genetic background and mutation effect, it is possible to tailor our care to individual the colonies we maintain. Structuring the observations we make with controlled language, key developmental time points and experimental load and linking these to our informatics solution we are now able to adapt our approach per colony and implement protocols that enhance the welfare of the animals we care for. Here we present how their utility may be further refined to meet the welfare assessments in GA models.

S11-4 Redesigning Health Monitoring: A Win-Win Experience for 3Rs and Resources

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Health monitoring is a major challenge in the modern animal facility. Currently, rodent health monitoring according to the FELASA recommendations implies the quarterly screening of populations. Moreover, comprehensive screening tests are quite expensive and require the euthanasia of the animals tested. The soiled bedding sentinel method seems to be the most popular sampling method. However, for this method to be reliable, a significant amount of bedding must be transferred from each cage. This translates into a relatively high number of sentinels per housing unit, even if a rotation scheme is implemented for those sentinels. In high-standard vivaria and/or when expected prevalence of infection is low, it is advisable to set both immunocompetent and immunodeficient sentinels per batch of sampled cages to account for more reliable serologic and microbiological screens, respectively. This approach, however, duplicates the number of sentinels used -sacrificed-, contradicting the 3Rs' recommendation for reduction. Recently, modern molecular methods for microbe screening have been developed that promise equal to better accuracy and sometimes a lower price when compared to the traditional methods. However, these new methods still present some limitations. Aiming for high health screening standards while optimizing resources in our SPF/SOPF rodent facility, we have redesigned the health screening strategy, moving from a conventional approach to a combination of traditional and molecular methods. This presentation summarizes the impact of this new, mixed strategy on routines, resources, and on the 3Rs.

S11-5 Potential Vertical Transmission: The Case of Mouse Norovirus

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Mouse Norovirus was first described in 2003 and it is now one of the most prevalent viral pathogen in rodent animal facilities. We report a natural infection of murine Norovirus in immunocompetent C57BL/6N

and immunodeficient athymic Nude-nu (nu/nu) mice. Virus was detected by molecular methods (RT-PCR) and the study was carried on for the duration of 1 year in which tissues (feces, mesenteric lymph nodes, intestine, spleen, liver, lung, brain, ovary and testis) from 3 males and 3 females of each strain were collected and analysed at 2, 4, 8, 12, 24, 52 weeks. Tissue distribution shows that all the organs tested were infected at various time and in particular that reproductive organs were positive at specific dates. Sperm derived from positive testis was tested and showed positivity after several washes, indicating that the virus strictly associates with spermatozoa. These spermatozoa were used to perform In Vitro Fertilization (IVF) and produce two-cells embryos. Embryos produced this way after a minimum of ten washes were infected but failed to transmit the infection to the foster mother after embryo reimplantation. Positive ovaries were used for ovarian transplantation without vertical transmission. Potential vertical transmission of mouse Norovirus through assisted reproductive technologies, especially the ones that soften or break the integrity of the zona pellucida, is under investigation.

S12-2 The Guide for the Care and Use of Agricultural Animals in Research and Teaching and Its Utilization to Provide Guidance in Agricultural and Biomedical Research

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The Federation of Animal Science Societies (FASS) is an organization composed of US and International scientific societies focusing on the use of agricultural animals in the production of food and fiber, and the use of these animals in research, testing, and teaching. FASS publishes the Guide for the Care and Use of Agricultural Animals in Teaching and Research (the Ag Guide) as part of its educational mission. The Ag Guide provides up-to-date information on science-based farm animal care in both agricultural and biomedical teaching and research. The Ag Guide was first published in 1988 and is currently on its third edition. The Ag Guide serves as a primary reference document for meeting the needs and requirements of agricultural animals utilized in research and teaching. It was adopted in 2011 by AAALAC International as one of its three primary references utilized to evaluate animal care programs. This reference resource is written in a manner that allows it to be easily adapted to a variety of agricultural animal management systems. Specific management practices that provide for optimal care, handling, housing, nutrition, and husbandry practices for the common agricultural animal species are detailed in numerous chapters. The Ag Guide provides an in depth overview of the requirements necessary to develop and maintain an effective institutional program of agricultural animal care and use. Utilization of a resource such as the Ag Guide establishes a harmonized set of guidelines for agricultural animal care at universities, government laboratories, and industry research facilities.

S12-3 Animal Welfare Assessment at Experimental Facilities in Dairy Cattle

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There is a general agreement that experimental animal's welfare should be assessed during research activity. The awareness that welfare is multidimensional requires a multicriteria evaluation resulted in the decision to base any animal welfare assessment system on four main principles: good feeding, good housing, good health, and appropriate behavior. The European Food Safety Authority (EFSA) recommended the use of scientifically evidenced animal based measurements for assessing animal welfare. They are likely to be the most direct reflection of their actual welfare state, permit to compare animal's welfare under different farming systems and remain more transparent to stakeholders. When building new research facilities, animal welfare should be thoroughly considered at the location, initial design, cattle acquisition, management and control. Settlings to the new unit should also cover management and emergency protocols, checklist and recommendations. Independently of the topic of research, any experimental design should assist dairy cattle welfare by meeting environmental resources on infrastructure and minimize climatic constraints with major concerns on thermal stress. Most critical obstacles firstly regard the milking and calving management since they might exert stress. Useful indicators for monitoring are kicking and stepping (milking) or tail-up position and looking at own flanks (calving). Particularities to milking and calving that affect profoundly the experimental design are different observation times and confrontation to stockperson or other cows. Finally, this overview also addresses research units under extensive management with certain preconditions that potential adversely affect an optimal status of welfare with less extent direct observations and cases of under recording.

S12-4 Management of Poultry in Research

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Chickens and other birds have been, and continue to be, used extensively as biomedical and agricultural research models. Poultry is considered a major global food source, being its production widely distributed all over the world. Therefore, several poultry species are used in experimental models to deepen our knowledge in poultry diseases and management. In addition, chickens are also important models in biomedical research, including cancer, genetics and toxicology. However, poultry includes a wide range of bird species and breeds, with distinct features and management requirements and therefore, animal facilities and management should be adapted depending on the species to be allocated. According to the level of biosecurity required for the experiment and the number of birds included, experimental birds can be allocated in isolator units, boxes, or conventional farms. In veterinary research SPF chickens and commercial layers and broilers are the most commonly used birds. Particular management conditions should be carried out in SPF chickens and layers because its nervous behavior that favors pecking. Also, partridges and quails are commonly used in veterinary research. Although not many specific necessities are required in the facilities, the expertise of the caretakers is essential to induce minimum stress to the birds. Finally, the use of wild birds as falcons, needs the enrichment of the experimental facilities to totally satisfy their requirements and reduce the stress, together with the training of caretakers and researchers which should be in contact with them.

WS03-1 Why Genetic Monitoring?

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During the past few decades of biologic research the numbers and diversity of mouse and rat strains available for studies has substantially increased. This is due largely because the means to genetically alter mouse and rat strains have greatly improved and become more available to the research community. With this explosive growth in mouse and rat strains the risk of using mice or rats that are genetically dissimilar from the presumed genotype has also substantially increased. There are a number of potential reasons why such a genetic mistake can occur. The ICLAS is developing a program that focuses on providing the means to mitigate the risks of genetic contamination of mouse and rat research strains and avoid the pitfalls of using a genetically inappropriate mouse or rat in an experiment. This talk will focus on the rationale for performing genetic quality control and how the ICLAS genetics program may help individuals and institutions to address these issues

WS03-6 Genetic Monitoring and Genetic Drift

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Steven Hawking said, "Without *imperfection*, you or I would not exist". Imperfections in DNA replication and repair have given us our existence, our species and the variation within our species. On the negative side such imperfections, or mutations also can be responsible for many diseases and genetic predispositions. When working with experimental inbred strains of rodents these continual occurring mutations leads to slow and inevitable genetic change or drift. These genetic changes lead over time to changes in phenotype and the animal response to its environment. Such changes in a basic research "reagent" are in sharp contrast to the need in science for defined reagents allowing reproducibility over time and place. Therefore as a community, in order to provide the nearest approximation of reagent grade experimental animal strains we need to understand and then control genetic drift. At the Jackson Laboratory we began to try and examine the nature and level of genetic drift occurring in inbred strains, and its potential impact. Next, we have begun to explore best practices for containing this inevitable process to enable researches access reproducible resources the world over. I will attempt to explain these points, using examples from our latest data on genetic drift in the commonly used inbred strain, C57BL/6J, including full genome sequencing and include the impact of implementing our Genetic Stability Program on cumulative genetic drift.

S13-1 New Project Development: Efficiency, Economics, and the Environment

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The past decade has seen the introduction of a range of national policies, initiatives, incentives, and penalties, as drivers for "sustainable development". Sustainable development aims to balance economic development and higher standards of living, now and for future generations, while simultaneously protecting both human health and the natural environment by reducing the use of natural resources and pollution. This requires a careful balance between economics, energy use, operational efficiency and the environment. For laboratory animal facilities, as major users of energy resources and waste generators, this is a particular challenge, particularly against a background of cuts in operating budgets. New approaches to facility design, equipment selection and changed behaviors are necessary and need to utilize a number of new approaches, models and metrics. The introduction outlines the issues while subsequent presentations in the seminar will identify the challenges, drivers and potential solutions in delivering "sustainable" new project development" in vivaria.

S13-2 Driving the Delivery of Sustainable Laboratory Facilities in the UK Higher Education Sector

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Animal facilities present a unique challenge for efficient and sustainable design and operation, with their inherent complexity of systems, intensity of resource consumption and waste production, regulatory compliance and long-term flexibility and adaptability needs. Drivers spurring on UK Higher Education Institutions to face these challenges include cost, European and national environmental regulation, tightening health & safety regulation, pressure from national funding bodies (which has resulted in a sector target of an 80% reduction in carbon emissions from 1990 levels by 2050) and student expectations (in part via a student-led ranking of institutions based on environmental performance). The Institutions response to these pressures has included a cross-laboratory Sustainable Labs initiative, bringing together Laboratory Facilities Managers, Laboratory users, Estates and H&S Professionals to prescribe drive and deliver best laboratory practice in a sustainable and economically efficient manner. The initiative draws on best practice guidance from the US Labs 21 and UK S-Lab programs and the laboratory aspects of the BREEAM and LEED building environmental assessment schemes. Action to reduce the environmental impact of animal and other Life Sciences and Medical Sciences facilities have been identified and implemented, for example, more efficient cold storage and more effective waste management. A major goal is modifying air change rates based on evidence that this will not impact on animal welfare but implementation requires convincing of the UK's rigorous inspection regime for animal research facilities.

S13-3 Waste Not: How the Paradigm Shift in Cagewash Equipment Can Significantly Reduce Energy Consumption and Reduce Operating Costs

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We are at a paradigm shift in vivarium technology, in which there are choices that can be made, particularly in cagewash equipment and methods, which can significantly reduce energy consumption space and operational costs.. This is particularly germane to cage-washing operations, equipment and methodology. Data from the USA indicates that the lack of exploration into alternate technologies and reliance on narrow previous experience can lead to millions of dollars in wasted water, electricity, steam and other energy resources. With ever-increasing energy costs globally, wasteful spending in these areas may result in less capital available for other research endeavors. However, exploring new options is a common challenge in new facility or renovation design. This presentation uses a year-long in-depth case-study, on applying a process-oriented life-cycle equipment cost evaluation procedure, to illustrate benefits, improvements, potential energy and cost savings to decision makers and to challenge the conventional notions about space allocation, and energy consumption in animal facility cagewash operations. These techniques provide: (i) a greater understanding of choices that can be made early in the design process to ensure that sustainable awareness and choices are brought to the table; (ii) tools for the evaluation of process, procedure and methodology of cage-wash operations to achieve time, energy and labor savings; and (iii) knowledge of simply adapted planning design principles and details which improve working environment in cage-wash areas within vivaria.

S13-4 Improving Animal Facility Efficiency Step by Step: Logistics and Lean

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The organization of flows of people, animals, materials and equipment is a key aspect to efficiently manage a laboratory animal facility. The authors manage a mouse facility holding around 6,000 IVC cages, which are integrally changed every two weeks. Cages are handled as micro-isolator sets in the barrier area and transported as complete setups to the washing area, for disassembly, emptying and then washing in a rack washer. Clean components are reassembled; bedding and feed introduced, and reassembled cages sterilized. The facility grew up over the years and new solutions were developed to improve safety, quality, and efficiency of operations. The first approach was the installation of a robotic system to automate the process of disassembling and emptying dirty cages, and the introduction of a new transport trolley associated with the system. This solution allows cages to be loaded onto transport trolleys in the holding room, brought to the washing area and loaded directly into the automation cell without any additional manipulation. The second step was the reorganization of the cage and bottle workflows using a 'lean' approach. Lean is a process management philosophy fo-

cused on maintaining or increasing value with less work and resources, eliminating waste. Cage and bottle workflows were balanced and timed around the autoclave throughput. Moreover, visual procedures with process layouts were defined. The two solutions synergistically contributed to optimize the facility productivity by smoothing the workflows and dramatically reducing the working hours dedicated to cage and bottle processing.

S13-5 Design and Development of a New Animal Facility at Trinity College Dublin: Opportunities and Pitfalls

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We outline our reasoning behind deciding to build a new multi-unit facility and choice of location. We currently have 6 units spread over a large area on campus and were given new space to consolidate 4 units into 1 area. We look at the vast improvements made with our new build, the ups and downs in the planning and building of an animal unit within a new building and the benefits and restrictions of choosing to build a unit at basement level. We look at going from separate units with independent working to units with central washing areas, a staff room and having Chief technicians on site, so they can better interact with both staff and researchers. We discuss how we came to our plans and how these changed as we moved from the planning stage to finished working units and the troubleshooting that needs to be done. The advantages and disadvantages of updating equipment built into the units are discussed. Are our animals better cared for in our new unit? Are they cleaner and can we keep them that way? Will it improve staff morale and lead to better research? We also look at the new techniques and tracking systems we included in our units. Finally, looking at staffing pre- and post-build, we investigate how we can best use our staff and the number of staff needed.

S15-1 Outsourcing Services in a Lean Mouse Facility

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The authors manage a 600 m^2 mouse facility currently housing around 6,000 ventilated cages dedicated to breeding genetically modified mice and for experimental activities (mainly oncology). The facility was opened in 2007 and the number of cages housed steadily increased together with the complexity of operations. Animal care activities have been outsourced since the beginning, taking advantage of the flexibility of this solution in adapting to a rapidly changing environment. Over the years, new technological solutions, such as a dirty cages automation system, were introduced to increase the productivity of the facility. Recently, a Lean transformation was implemented to further improve efficiency. Lean production is a management philosophy derived from the Toyota Production System, which considers the expenditure of resources for any goal, other than the creation of value for the end customer, to be wasteful, and thus a target for elimination. The results of the Lean transformation exercise were striking, reducing, by up to 50%, the working hours dedicated by the animal care staff to most of their activities. However, the introduction of Lean management represented a substantial challenge for the entire staff of the facility. This experience will be discussed in details together with the challenges encountered during and after the transformation with a particular focus on the outsourced services.

S15-2 A Third Option: Outsourcing + Indoor

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Our animal facility provides services to 6 research institutions located in the same building. 1500 professionals are organized in 100 research groups working in different biomedical fields. It is a 5000 square meters facility with 6 independent units with common working procedures for all the users. The objective of this presentation is to share the organizational chart of the facility and how responsibilities are distributed. A combination of indoor and outsourced animal facility management system is used. A team of 32 professionals are working in the animal facility. The direction, management, supervision, veterinary care and animal facility administration is from our institution (10) while animal technicians are outsourced (22). On the one hand, responsibilities like resources, equipment acquisition, standard procedures and scientific advice come from the animal facility direction. The daily operations where other departments are involved, like maintenance, health and safety issues, human resources and quality systems are coordinated through the manager. The supervisors act as a bridge between the researchers and the animal facility, working as customer services. Some of the supervisors are veterinarians, who are in charge of veterinary care and animal welfare of each of these units. On the other hand, animal technicians are organized in teams by animal rooms and units. There is a coordinator, who is in charge of planning the weekly staff calendar and ensures the quality of the work. He is the connection between both companies. This system allows providing a high quality animal facility service, with highly qualified staff without losing company culture.

S15-3 Right-Sourcing Your Animal Care and Use Program

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The trend to outsource services has been growing during the last years in the biomedical research and drug discovery industry. Outsourcing services not considered essential for an institution helps managing resources in a flexible and efficient way. However, outsourcing services considered essential (core) is not regarded as an ideal solution for scientific, strategic, logistical or organizational reasons. For these critical services, insourcing can be the right sourcing option. For most research institutions, the Animal Care and Use Program (ACUP) is a core resource. ACUPs must adjust operations to changes in demand. In a rapidly evolving environment, it is essential that ACUPs quickly adapt to new models, research protocols, experimental techniques, staff training and education requirements, reporting and record-keeping systems, etc. Furthermore, implementing new technologies and complex regulations claim additional resources and staff. An insourcing/outsourcing model for the ACUP will bring value to an institution, but it will present challenges (staff integration, supervision, communication, etc.), which must be properly managed in order to avoid problems and allow an optimal service provision. Which is the best sourcing solution for your ACUP? Unfortunately, there is not a single solution that fits all services and facilities. Features such as size, complexity, type of species, research projects, nature of the animal protocols, etc., influence the design of the best strategy for each individual case. The aim of this talk is to discuss key elements to be considered during the design of the insourcing strategy, which will best meet the requirements of different ACUPs or facilities.

S16-2 Occupational Health and Safety Program in an Animal Imaging Facility

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In vivo imaging techniques are attractive preclinical research tools because they are minimally invasive and longitudinal studies can be carried out. However, risk assessment becomes a key and complicated issue, especially at the boundaries between different areas. A fully

equipped in vivo molecular imaging facility offering services in PET, SPECT, CT and MRI should have a radioactive facility, an animal housing unit and an MRI unit. Three invisible risk factors (radiation, pathogens, and strong magnetic fields) coexist in a confined area, while the regulatory frameworks and OHS guidelines which may apply to every individual aspect might collide to the others. Therefore, an appropriate design of the whole facility, the implementation of SOPs, the establishment of an appropriate training program and the adoption of certain compromises are essential features to guarantee operational health and safety, while the performance of the whole facility is not constrained. In the present lecture, the main aspects related to OHS issues in the pre-clinical imaging facility at CIC biomaGUNE will be discussed. The facility design, the implementation of physical barriers, the different dressing codes, the training program and the different compromises adopted during the three years of operation will be discussed.

S16-3 Occupational Health and Safety Issues Working with Animals under Biosafety Conditions

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A high-biocontention facility where it works with experimental animals inoculated with pathogenic biologic agents of level 3 (BSL3), must have as its main objective the implementation of functional and technical tools that allow monitoring and control of all scheduled performances ensuring an acceptable space of individual and collective security. To cope with success guarantee the occupational health and safety of the animal facility workers, should act simultaneously on two different fronts: firstly, the collective security that is provided by a correct and functional design, the establishment of a negative pressure with respect to atmospheric in unidirectional differential gradient, implementing biosafety absolute filters element of the treatment of air supply and exhaust and effective treatment and control of effluents and solid waste generated in the animal facilities, secondly the personal security guaranteed by the implementation of a program to monitoring hygienic indoor environment against to the presence of biologic and chemical contaminants and the adoption of correct personal protection.

S17-1 Setting Up a Zebrafish Facility

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Zebrafish is a live research model that nowadays is getting a great development for the last ten years. Aquatic animals have a high dependence of the environment, so the main job in these facilities is to control and keep the environment in the correct parameters. To make this control we will need a lab to check the parameters and modify them. For this reason the design of the LSS (Life Support System) and the maintenance will have a lot of importance because we will have to adapt our routines for their specific design. This animal has a non-direct development so we have to adapt the food to the development stage using different types of dry and live food (the nutrition protocols differ a lot from one facility to another one) so we can assure the right development. Another thing to take in account is the health status, the animal provider and the exchange rate with other facilities. There are not SPF fishes, but in the last years a lot of tools have been developed like the PCR's. These subjects should be standard in the different facilities so its time to develop a strong professional net, that standardizes all these matters.

S18-1 Managing Laboratory Animal Facilities with the Right Attitude. Create a Management Culture that Works

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Managing animal facilities is getting more and more challenging, especially due to higher demands from users and investigators using genetically modified animals. A lot of effort has been put into planning and building new laboratory animal facilities, but less effort has been put towards understanding the impact of an efficient management of an animal facility as a whole. Lately, laboratory animal care programs are being challenged to do more with less, as the economic pressure forces institutions to look more into money spent for the operations. This has been an expectation of managers in other industries for decades. Proper management techniques applied in daily running of well planned and built animal facilities will lead to better motivation of the workers and more effective running of the facility. Significant savings in yearly running costs can be achieved. This talk will introduce some of the management concepts that have been adopted with success in leading companies in many other business sectors. Also, the concept of enabling animal care staff to take over the responsibility of their own work to become smarter, more independent, and better motivated work force will introduced. But most importantly, this talk will introduce the "Right Attitude" tools, such as "lean management", can only work if you have the management culture allowing them to work. And at the end of the day, it's all about the Right (positive) Attitude!

S18-2 Human Resource Management in a Transgenic Core Facility

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The Transgenic Core Facility (TCF) at the Max Planck Institute of Molecular Cell Biology and Genetics (MPI-CBG) in Dresden, Germany, was founded in 2002 as an internal mutant mouse service. As an academic non-profit facility, the TCF has successfully covered all operating costs since 2005, and has opened its doors also for an external service for universities and academic institutions throughout Germany and Europe. The team of TCF currently consists of one leader and three technicians. We have intensively developed our own strategies of employee involvement, such as: spontaneous technology development, project management, teaming for special projects, team meetings with rotating chair, constant efficiency improvement, staff appraisals, social events planned by the staff, professional personal development (courses, workshops), as well as intensive and consistent connection with the animal facility personnel. In my talk I will show how the relatively small team can yearly generate about 35 transgenic mouse lines with about 250 founders, 25 ES-cell projects with about 200 chimeric mice, hundreds of rederivations via embryo transfer, as well as freezing hundreds of strains down for a backup. This efficiency can only be possible with a fantastic spirit between the team members over the years. I will show in my talk that people and their attitude can make the difference.

S18-3 Consider the Welfare Not Only of Animals, But of People and Machines as Well in the Vivarium

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The field of human resources implies and includes the aspects of welfare and training of persons. As the current president of LAWTE (Laboratory Animal Welfare and Training Exchange, I am concerned about such issues because of the implications to the research animals. To extend the thinking, a laboratory animal trainer should recognize and promote welfare overall. What an animal care provider feels and knows is likely to affect the conduct of his/her job working with animals. Using the purchase of a piece of equipment as an example, the talk will address factors related to human resources in the vivarium such as ergonomics, people welfare, costs including money and frustration,

animal welfare, product ease of use, engineering and performance standard expectations (like the United States ILAR Guide or the AAALAC accreditation program would evaluate), and sustainability. In a sense it's the life cycle analysis (LCA) concept often used with machines, but applied here more broadly to additional components that are engaged in conducting research in the animal facility.

S19-3 Sterilization of Blower Units in a New ABSL 3 Containment Using Hydrogen Peroxide: Cycle Development and Validation

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Before putting an ABSL 3 facility into operation, it is a legal requirement in Germany to have a validated concept for efficient decontamination of all rooms and types of animal and lab equipment. This is equally important in case of an unwanted accidental contamination during operation of the unit and also on a regular basis prior to conducting maintenance work. Cages and racks can be easily sterilized by autoclaving, whereas electronic equipment like blower units can only be decontaminated by other methods like spraying or fumigating with chemical agents. The aim of this study was to develop and validate a sterilization method for blower units of a special ventilated caging system in a new ABSL 3 containment unit. Four blower units were placed in a room of 61 cubic meters. The hydrogen peroxide was equally distributed in the room via the HVAC system by connecting the generator to the ventilation pipes. The authors validated a 3 hours fumigation cycle using 1900 ml vaporous hydrogen peroxide. The efficiency was monitored by chemical and biologic indicators put in 6 different locations in each of the 4 blower units. All fumigations were carried out three times. A 6 log reduction could be achieved in all 72 spore samples as proved by spore strips of *Geobacillus stearothermophilus*. This method is reproducible, easy to use and very effective for sterilizing valuable, heat sensitive animal equipment like blower units. There are no signs of corrosion and functional damage after more than 5 fumigation cycles.

S19-4 Doing the Right Things the Right Way: The Importance of Safe and Secure Research

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Researchers are confronted with a rapidly expanding scientific literacy gap between themselves and the general public. The more complex science becomes, the more disengaged the non-scientific community becomes. The more disengaged the public becomes, the less likely the public is to support research. The less likely the public is to support research, the less likely we will be able to solve the most pressing health issues that we face. The gap, unfortunately, often places fears and misconceptions beyond facts and such fears and misconceptions often result in legal environments that make research difficult. This presentation highlights how research communities can build trust with the general public through demonstrating obvious efforts to mitigate risks and creating a culture of responsibility that builds trust with the global community. The new needs of research not only include results from research, but also sound demonstration that scientific research is a vital component of the advancement of human and animal health. Jim Welch is the executive director of the Elizabeth R. Griffin Research Foundation, a non-profit foundation headquartered in the US and a major global advocate of safe research and for advancing the professionalism of the people who work therein.

S20-1 Transportation of Laboratory Animals: Research, Welfare and Legal Aspects

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Transport of laboratory animals by experienced, competent and highly-equipped companies using the shortest possible routes is crucial for both animal welfare and research. It is important for the scientific community, for authorities and for transport service providers to understand that transport of laboratory animals is an essential component of the success and quality of biomedical research in Europe and globally. Equally, lack of access to appropriate transport results in reduced animal welfare. The transport of live animals for use in research is the target of continued campaigns by animal rights activists. As a result, a significant number of European and international transport companies no longer transport live animals for research purposes. Transport in good conditions decreases animal stress and health risks during transport, and secures availability of healthy animals for research. The quality of animals impacts on the quality of scientific outcomes and therefore meets the need to use fewer animals per study. The unintended impact of the moratoria on transport is twofold1) Most animal models are only available in a few countries therefore moratoria on transport of certain species of certain air carriers and/or airports limit their availability for European research, which often means delaying or relocating research projects.2) Using less frequented routes and airports result not only in longer transit times and inadequate connections but equally impacts on availability of specifically trained staff and equipment ultimately jeopardizing animal health and welfare. The resulting delays and welfare issues are to be added to those caused by indirect transport routes.

S20-2 Continuity of Supply: A Wake up Call

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The Life Sciences sector in Europe faces unprecedented pressures on its animal research supply chain. The closure of all surface routes to transport animals for biomedical purposes across the North Sea and English Channel, and the continued shrinkage in the number of global airlines prepared to transport research animals, will inevitably impact on the long-term feasibility of Europe as base for key medical research studies. Imported animals are small in number but are both research and business critical. The reduction in choice of transport routes has meant that essential time-critical work is being displaced out of Europe; studies are being cancelled. If studies are routinely shifted abroad this will impact on R&D investment decisions by companies, placing the future of Europe as a key site for R&D in jeopardy. In recent years activists and extremists have developed tactics, which are increasingly effective. Using effective and smart strategies, combining intelligence, communication social media and networks, policy and legal actions in addition to more traditional campaigning (and criminality) they have developed a rich tool box of abolitionist tactics. In contrast we have witnessed a euro-wide reduction in pro-active communications and activities on the benefits of animal research. Unless we reverse this retreat we will see continued pressure on the supply chain and an inevitable flight of R&D out of Europe.

S20-3 Relevant Legislation and Guidelines

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Long distance and cross-border transport of any living animal implies, *per se*, zoonotic, zoosanitary, phytosanitary, economic and animal welfare risks. The transport of laboratory animals, while obviously having to comply with all the general requisites, must additionally face the challenge of some particular issues: Great variety of species; Most lab-animals are purpose-bred with a defined physiologic, metabolic, genetic or pathogen free status; Complex and non-harmonized regula-

tions; Increasing difficulty in finding professional carriers. It is obvious that the transport of lab-animals is quite complex task and requires a great deal of paperwork and specialized legal, veterinary and technical professionals. In what respects the EU, Directive 2010/63 imposes restrictive husbandry conditions and we expect to see a trend to import more animals. It is therefore more important than ever to establish a clear framework for the out-sourcing and cross-border movement of lab-animals if we want to preserve the health of our population, the biodiversity of the world we live in, the health of our livestock and of our wildlife and, last but not least, the welfare of the animals that are shipped. This presentation will present the most relevant legislation and guidelines affecting the transport of lab-animals (at EU level, OIE's Terrestrial Code, IATA's LAR, the Council of Europe's or ILAR-Guidelines). The major aim of this presentation is to disseminate the knowledge of regulations and non-binding guidelines and thereby contribute to foster harmonization at a global scale.

S20-4 An Integrated Strategy to Support Airline Companies

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The supply chain of laboratory animals including international exchanges is a key point of the future of research worldwide. Good transport conditions and transfer are requested for ethical, safety and scientific reasons. This can only be achieved by securing the activity of hauling companies which is currently jeopardized by the attacks of animal rights activists. This concern does not address primate transport only, but also dog and rodent transport. In the absence of support from the European and National authorities to this legal activity, research defense NGOs to develop pro-active strategy to support transport companies. Different initiatives exist in that field especially in the US and at a lesser extent in Europe. These initiatives are mainly based on petitions and press articles demonstrating that transportation of lab animals is legal and done in good conditions. We will present here an example of integrated strategy developed by Gircor in France to support Air France-KLM. This strategy combines: * Direct support to the company by giving information on animal research * Active presence in transport organizations meetings * Actions towards national authorities, local service providers and animal care staff to develop a new international entry point in Roissy airport. This strategy seems to have been an effective support to help the company to feel more comfortable in maintaining the transport of laboratory animals.

S20-5 The Importance of Optimized Global Transport of Nonhuman Primates

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Most nonhuman primates used in biomedical research will undergo transportation at some point in their life. All transport of nonhuman primates should be in a way that does not jeopardize the wellbeing of the animals and ensure safe arrival in good health with minimal distress. The need for nonhuman primates in research has been justified by many stakeholders, and despite best effort, alternatives are unlikely to be found within the foreseeable future, hence maintained well-organized, safe and short-duration transport is critical to minimize disruption to the transported animals' welfare and to ensure future quality of research. As many nonhuman primates are bred in Asia or Mauritius, and typically used for research in countries in Europe or US, the total journey time can vary with different stages between departure and arrival at the final destination due to waiting, transit, unloading time and the risk of delays, which can all compromise the health and welfare of the animals, if not optimally planned. The increasing number of animal rights group activities against global animal carriers, including airline, ferry and shipping companies, aiming to force the carriers to stop animal transport and ultimately animal research, can impact the health and welfare of the transported animals as routes longer than geographically or logistically necessary with increased connection stages and potential delays need to be used. Further, this can compromise the quality of research and negatively impact medical progress for many life-threatening or debilitating diseases, representing a global issue for biomedical research, irrespective of geography.

S21-1 ISO and GLP Quality Management Systems in the Context of Regulatory Requirements

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ISO and GLP are both Quality Management Systems aimed at ensuring that an organization or product is consistent. They have however different scopes, while ISO is devoted to setting international standards covering almost all aspects of technology and business, GLP is focused on the quality of non-clinical safety tests. They also have different origins, while ISO originated in the 1940's when different countries got together to promote industry standards, GLP originated in the 1970's from the need of the FDA to prevent scientific fraud that affected consumer safety. Nowadays, ISO is a big international organization that promulgates proprietary standards, and GLP is a set of guidelines published by the OECD that adapt the requirements of the FDA to regulatory agencies outside the USA. The first step to be taken prior to deciding the implementation of a Quality Management System is putting them into context.

S21-2 GLP and AAALAC: Competitors or Partners? Opportunities and Challenges of GLP Certified and AAAALAC-Accredited Organizations

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The enormous benefit for having an animal care and use program AAAALAC-accredited goes without saying. Along with meeting all applicable local and national regulations, AAAALAC-accredited institutions must also demonstrate that their quality and animal welfare standards go above and beyond what is required by law. However, many institutions face the need to implement other quality management systems like Good Laboratory Practice (GLP) or International Organization for Standardization (ISO) 9000:2000. In these times of restricted resources and efficiency measures, it is not easy for executive management to decide whether or not the added value of a voluntary accreditation outweighs the efforts and investments required. This presentation summarizes what convinced/obliged executive management's decision to apply for both, GLP certification and AAALAC accreditation, it describes the challenges and opportunities faced during the process, resources needed (time, training, administration and synergies of two parallel quality management systems), lessons learned and the overall benefit to have both, GLP certification and AAALAC accreditation. While the AAALAC accreditation does not substitute for a GLP certification and the required inspections, and while it is no guarantee for automatic compliance with the applicable GLP sections, this presentation depicts potential synergies and advantages in the application for both, the GLP certification and the AAALAC accreditation.

S21-3 AAALAC Accreditation in a University Environment

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October 2000, the first academic central laboratory animal facility (ACLAF) outside the USA was AAAALAC-accredited in the Nether-

lands. The ACLAF housed rodents and farm animals and facilitated a wide range of research for a biomedical University including a large academic hospital. The ACLAF was founded by the University Board, which decided the diversity of researcher-run lab animal facilities were to be replaced by one state of the art facility which was up and running around 1990. This process paralleled the start in 1977 of the Dutch legislation on the use of laboratory animals. Professionalism, laboratory animal science & animal welfare, transparency for legal and researchers' requirements, and 'value for money' were key factors for all stakeholders involved: the University Board (create a centralized facility for top research, assure legal requirements are met, charge running costs to researchers), researchers (from own facility to 'customer', fear for freedom/flexibility, costs animal research visualized by invoices), and the staff of ACLAF (reallocated from the multitude of smaller labs, responsible for quality, flexibility and costs). The ACLAF AAALAC accreditation was preceded by ISO-9001 certification and followed by GLP registration. ISO for quality management and customer oriented structuring of processes, AAALAC for an independent evaluation of appropriate and humane animal care and use by international experts in laboratory animal science, GLP upon request of specific researchers. In this complex situation including political, management-, and laboratory animal science challenges, the AAALAC accreditation process was helpful to structure oversight and motivate staff, from Director to Animal Caretaker.

S21-4 Continuous Improvement of Quality in Animal Care and Use Programs: Strategies for Optimizing Animal Welfare and Research Using Quality Improvement Techniques with Performance Standards

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One major goal of the AAALAC International accreditation is to improve the quality of animal care and use through self-assessment by internal and external reviewers using published performance standards and guidelines. Quality of animal care and use can be defined by deciding which criteria or standards are Critical to Quality (CTQ) and establishing an auditing system to review whether or not a specific performance criteria or standard has been met. For example, institutions can establish performance standards from published standards such as the Guide, FELASA guidelines, and national regulatory requirements and define which criteria are CTQ which should be used in concert with sound professional judgment. Formal analytical processes and procedures used in quality improvement such as Root Cause Analysis (RCA), Corrective Action/Preventive Action (CAPA) and six sigma can all be successfully used to drive continuous improvement of quality in animal care and use programs. Important indicators of quality such as biosecurity, animal health monitoring, genetic monitoring, and error rates in animal care service/support can be used to measure and improve the quality of animal care toward a performance standard. Approaches of how to improve animal care and use quality for both research institutions and animal suppliers will be presented. An overview of how these quality improvement methods can be used in conjunction with other systems linked to quality such as ISO and GLP will be reviewed and how they may be used in an animal care and use program to achieve AAALAC International performance standards will be discussed.

S22-1 When Is It Animal Testing Morally Acceptable?

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The new EU directive came into force on January 1st, 2013. For many countries, compliance with this directive demands a dramatic overhaul of current practices. A committee will have to be created to judge the moral admissibility of the entire research project and an organization must be set up to determine and monitor the welfare of the animals being tested upon. My presentation focuses on the work of the first committee. How does one go about determining the moral admissibility of a research project in which scientific tests are conducted on animals? What morally relevant information should such a committee have at its disposal? In the Netherlands, we have a long tradition of committees that pass moral judgment on intended animal testing. I myself have been a member of several such committees for some time now. This practical experience has served as the basis for an assessment model that I have conceived together with other ethicists. My model is, I believe, sufficiently precise in explaining just how the importance of the research project can (or must) be weighed against the suffering of the animals concerned. This model is underpinned by theories derived from the field of ethics. I hope my presentation will convince the people present here today of the value in applying a uniform assessment model within the EU.

S22-2 Overview of Project (Ethical) Evaluation in Europe under the Directive 2010/63/EU

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The ethical evaluation of research projects or protocols has been traditionally performed in a variety of ways across Europe… when it has been performed. Under the Directive 86/609/EEC, there was no legal mandate to perform such evaluation. Several countries had issued national regulations that included the requirement for the ethical evaluation of research proposals, while in the other countries with no such legislation, the evaluation could be performed due to reasons such as institutional commitment, good practice, and funding or accreditation requirements. The systems in place were of different nature, and included participation of local and/or government bodies. The Directive 2010/63/EU seemed to be a way to harmonize this practice. However, this has not been the case. The Directive gives the competent (public) authority, and not the institutional Animal Welfare Body (a figure required in the Directive) the responsibility to perform the project evaluation. But Article 59 allows Member States designate bodies other than public authorities for the implementation of specific tasks laid down in the Directive. This means that the project evaluation, as one of the tasks laid down in the Directive, can be performed by other "designated bodies". This has resulted in some Member States allowing the designation of other bodies, including the Animal Welfare Bodies as competent authorities to perform the project evaluation. Therefore, the final scenario is heterogeneous again. The differences may not relate only to the nature of the bodies responsible of the evaluation, but also to their composition and expertise. The situation is discussed.

S22-3 Diversity in the Implementation and Organization of Project Evaluations across Some European Countries: Round-Table Session

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With implementation of the EU Directive 2010/63 there was the potential for greater consistency across EU member states in the implementation of a variety of standards and processes including those relating to the area of project evaluation. Before any project is granted part of the evaluation includes assessing the likely harm to the animals versus the expected benefits to human beings, animals or the environment in order to assess whether the use of animals is justified. The Competent Authority has the accountability for this 'harm-benefit' analysis although the Directive allows for other Designated Bodies to do this and this has the potential to lead to differences in the implementation of project evaluation. For many member states the legal position in early 2013 is still not clear. This session will focus on describing the

organizational operation of project evaluation in the UK, Netherlands, Spain, France and Germany by addressing the points below: (i) which body is defined as the Competent Authority; (ii) how is the evaluation process organised; (iii) how is impartiality assured; (iv) role of a National Committee. For example, in the UK, the Government Home Office is the Competent Authority with accountability for project evaluation, although the Animal Welfare Body is likely to have a role in reviewing projects from a local perspective as outlined in the draft legislative guidance. In France and Spain the project evaluation is delegated to ethics committees. Despite the different processes are there similar outcomes?

S23-1 It Is Possible to Predict Semen-Fertilizing Ability after Cryopreservation?

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Semen cryopreservation is considered as a useful tool in the genetic and reproductive management of experimental farms. In addition, embryo production laboratories with both commercial and experimental aims often rely on the availability of different batches of cryopreserved semen. In both cases, semen-fertilizing ability after cryopreservation has to be evaluated prior to its use. Currently, the assessment of sperm function in a raw or processed semen sample is not able to reliably predict its fertilizing ability. Therefore, the combination of different sperm function tests is needed to guarantee a more accurate prediction of thawed semen quality. This procedure is often expensive and time-consuming. Research attempts are thus focused on the development of reliable tests to predict semen quality and a battery of analyses have been investigated in different domestic species. In vitro fertilizing ability is the most adequate parameter for semen fertility evaluation. On the other hand, this test depends on the availability of female gametes and it needs to be repeated with several batches of oocytes to avoid the effect of their quality. Spermatozoa DNA integrity and ATP intracellular levels represent two factors able to strongly influence in vitro and in vivo embryo outcome with frozen/thawed semen and have been linked to embryo production in different domestic species. In addition, they can be evaluated both prior and after freezing, thus providing data on spermatozoa cryotolerance. Oxidative status by enzymatic assays is also being evaluated as an alternative parameter of sperm quality both before and after cryopreservation.

S23-3 State of the Art of the Cryopreservation of Fish Sperm

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Fish sperm cryopreservation is a technique of undoubted interest that can be applied in several fields of research and production. This technique is well established in freshwater and marine fish species. For aquaculture production, sperm cryopreservation allows synchronizing gamete availability and the transport of sperm within fish farms. For research, cryopreservation provides advantages by allowing germplasm storage in genetic selection or conservation programs and mostly important by simplifying broodstock management in research facilities. Nowadays, several species are being used as research models not only for aquaculture development applications, but also for medical research. Sperm cryopreservation can give an important contribution in the germ storage of transgenic lines, reducing the number of animals in experimentation, costs and risks of being accidentally destroyed. Our group has been working in the last 15 years in the development of cryopreservation protocols for commercial and endangered species. Our main interests have been the use of cryopreservation in broodstock management as a source of material for research purpose. Recently, seabass sperm cryopreservation protocols allowed a correct storage of cells for further analysis of antioxidants incorporated through the diet, being able to reduce the number of breeders in experimentation and determine the relevance of incorporating such substances in animal feeds. Fish sperm cryopreservation can be also used as a biomarker for contaminant analysis, avoiding the sacrifice of animals during experimentation. In this talk, several aspects of fish sperm cryopreservation will be discuss regarding their applicability in research, focusing on the benefits of this tool in several fields.

S24-1 Disaster Plan and Crisis Preparedness

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In biomedical research, the use of laboratory animals, in addition to *in vitro* testing ad clinical research, is essential. As this is a privilege and a responsibility, processes and procedures for the daily routine practices should be in place to ensure the most appropriate care and high standards for the use of animals. Such accountability is to be considered 7 days a week. But animal facilities could face unexpected events. Contingency plans should be prepared to address natural disasters, failure of critical systems, significant personnel absenteeism, or criminal activities. Although those conditions are not frequent or unlikely, they are disruptive for the activities and detrimental for the personnel and the animals. Disaster plan, including risk assessment, preventive measures, preparedness and crisis management, should be established in conjunction with the management of the animal facilities, the research team, the HSE staff, and any other appropriate stakeholders; it should be approved by the responsible of the user establishment. To be effective, the personnel should be informed and trained about the details of the plan to have rapid and suitable reactions. It is recommended to perform large-scale exercises with the personnel in order to test the plan and to assess its performance.

S24-2 Don't Panic! Dealing with the Disaster

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Despite sophisticated barrier controls, one of the most common 'disasters' in the animal facility is the detection of disease or infection. Underpinning the concept of 'disaster planning' is a clear definition of 'disaster'. A disaster may be any unwanted organism and depends upon the goals of the animal facility and the research that it supports. A 'disaster plan' must include pre-existing safeguards, for example, robust health monitoring programs and risk-management procedures. It also has to be assumed that a 'disaster' has been detected and confirmed by some or all of the following: the appearance of clinical signs in animals, routine health monitoring, phenotypic variation, or unexpected experimental data. Lack of preparedness can turn a disaster into a catastrophe. One cannot always anticipate the unexpected, but having a plan ready for the event, and subsequent recovery, is paramount. Communication of the plan in advance is critical, and all appropriate personnel must be aware of it. Common disasters include Mouse Hepatitis Virus (MHV), pinworm, Parvovirus and may also include -depending upon the barrier quality- *Pseudomonas aeruginosa*, *Klebsiella* spp and *Staphylococcus aureus.* There are also examples of outbreaks of disease considered to be rare, such as Ectromelia. It does not follow that a 'disaster' must result in depopulation. A clear idea of which agents will be tolerated, which may be tolerated temporarily and which must be immediately excluded will provide a defined pathway in the event of an outbreak. Panic can be averted by attention to 3 watchwords: isolate, react, communicate.

WS05-1 Design of Animal Registration Software: One Size Fits All, Tailor Made or Somewhere in Between?

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Animal registration, breeding logs, transgenic data, licenses, legislation, certification, etc. how do you keep track of all that information. Pen and paper are still reliable tools; Excel sheets are more practical, but have their limitations; and then there are software packages or 'solutions' as they are advertised. 'Solutions' for our animal management requirements and needs! A genuine question is: what do we need to manage? Some aspects are of a general nature, are found in most if not all facilities. Others are more specific for a particular country, like the registration requirements, or for a particular facility. In this workshop: requirements, demands and dilemmas are presented from the animal management point of view. Suppliers of animal management software will present their solutions to those requirements, demands and dilemma's. You are encouraged to share your views and experiences.

S25-1 Hearing Disorders in Aged Mice

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Age-related hearing loss (ARHL) is a universal feature of mammalian aging and is the most common sensory disorder in the elderly population. The molecular mechanisms underlying ARHL are unknown, and currently there is no treatment for the disorder. ARHL is associated with the progressive degeneration of cochlear sensory cells, spiral ganglion cells, and/or stria vascularis cells. Several mouse models have been produced to mimic the different forms of human ARHL. The C57BL/6J mouse strain displays the classic pattern of ARHL by 12 to 15 months of age, which begins in the high frequency region. These functional deficits are accompanied by the loss of hair cells (that begin in the base and spread toward the apex of the cochlea with age) and neurons. Senescence-accelerated strain prone 8 (SAMP8) mice suffer an earlier development of cognitive age-related pathologies and a shorter life span than conventional mice. Recently, it has been reported SAMP8 strain displays early hearing loss with the sequential degeneration of outer hair cells (OHCs), spiral ganglion neurons (SGNs), stria vascularis and ultimately inner hair cells (IHCs), which mimic human presbycusis. The pronounced reduction in endocochlear potential (EP) results from stria vascularis and fibrocyte damages. The fact that SGN loss occurred before IHC death indicates that degeneration of SGNs is not a consequence of hair cell loss. Together, these discoveries indicate that the SAMP8 model could be a valuable tool to investigate the molecular mechanisms responsible of ARHL and to develop the effective treatments.

S25-2 Caring for Aging and Aged Nonhuman Primates

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Nonhuman primates have been used as laboratory animals for more than 50 years. Species may vary from small nocturnal lemurs to chimpanzees, from wild caught to captive bred. In captivity these animals may reach up to 50 years of age. Based on their close genetic homology to humans they serve as translational models. Aging is an inevitable process in all organisms accompanied by loss of normal physiologic functions and degenerative and chronic diseases. Research in aging nonhuman primates may require care and monitoring of wellbeing for decades. In addition it may be challenging to meet the social needs of these animals. Adequate health surveillance and disease prevention is essential. Speaking of non-domesticated species, animals tend to mimic symptoms of pain, distress and discomfort, and examination may require manual or chemical restrain. Diseases of aging monkeys range from metabolic diseases, nutritional disorders, gynecologic problems (for example, endometriosis), tumor development or abnormal behaviors. Appropriate humane endpoints maybe study specific but it is essential to include psychologic and behavioral symptoms.

S25-3 Growing Zebrafish: A Challenge?

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Throughout the years, zebrafish (*Danio rerio*) takes on an important role in a variety of biomedical projects, not only in basic research. The high reproduction rate, combined with the inestimable value of transparent extracorporeal development stages in a short time period, make this species so interesting. Because of their small size and the high spawning rate it is possible to maintain a large amount of animals in a relatively small facility. Nevertheless the technical set up for the housing conditions differs completely to standard housing concepts from traditional laboratory vertebrates and is essential for raising zebrafish successfully. In addition to a well-working water treatment plant and an adequate feeding plan attentive, skilled and highly motivated staff plays a major role when handling fish.

S26-1 Generation of New Animal Model for Alzheimer Disease by Interfering with Synaptic Protein Complexes

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In the past decades, genes causing familiar forms of Alzheimer disease have been identified and provided the genetic framework for the emerging amyloid hypothesis. On the basis of these findings, engineered mouse models have been developed and have allowed the understanding of crucial information about the pathogenic process. Certain observations obtained by transgenic mice, however, do not easily fit with the simplest version of the amyloid hypothesis. Even if there are transgenic lines that offer robust and relatively faithful reproductions of a subset of Alzheimer disease's features, a mouse model that recapitulates all aspects of the disease has not yet been produced. We describe here an innovative, non-transgenic animal model of Alzheimer disease. This model mimics early stages of sporadic disease, which represents the vast majority of cases. The model was obtained by interfering with the complex between the disintegrin and metalloproteinase domain containing protein ADAM10, the main alpha secretase candidate, and its synaptic partner SAP-97. Association of ADAM10 with SAP97 governs the enzyme trafficking and activity at synaptic sites. Interfering with ADAM10/SAP97 complex in vivo for 2 weeks by means of a specific cell-permeable peptide is sufficient to shift the metabolism of the amyloid precursor protein towards amyloidogenesis and allows for the reproduction of the initial phases of sporadic disease. After 2 weeks of treatment we detected progressive Alzheimer disease-like neuropathology. Behavioral and electrophysiological deficits were also detected.

S26-2 Progress in Translational Animal Models of Parkinson Disease

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There is a long history of modeling Parkinson disease in the rodent and primate but this has largely focused on the dopaminergic depletion and resulting motor deficits that were established as the primary characteristics of the disease several decades ago. However, it is now well recognized that non-motor dysfunction and non-dopaminergic pathology may also play critical roles in disease development. These aspects of the established models are now being characterized and that process includes both of the two main types of models used to date. Using toxins to target damage towards the nigrostriatal dopaminergic innervation and, more recently, genetic models exploiting the growing knowledge of the role of genetic susceptibilities in Parkinson disease

provides us with a large range of models at our disposal. However, disease modeling alone does not suffice in Parkinson disease, which also requires recapitulation of additional elements, such as the therapy-induced side effects which remain a significant concern in the treatment of the disorder. Key in Parkinson disease research therefore, is the selection of the most appropriate model for the task in hand and recognition of its strengths and limitations in addressing that question.

S27-1 Mouse Models of Neurodegenerative Diseases: Behavioral Approach

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Neurodegenerative diseases have probably been, for many years, one of the most important challenges in the neurologic pathology. All of them have in common their progressive and irreversible course; however, differ in their clinical manifestations, such as early dementia in Alzheimer´s disease, motor disorders of Parkinson disease, or muscleatrophy in amyotropic lateral sclerosis. One of the most recent experimental approaches and that has proved to be an excellent way to address the study of the causes and mechanisms of these diseases (in addition to studies on primates) are genetically modified murine models and, more specifically, mouse models. These models allow experimental approaches to the etiology of several human diseases because of their phylogenetic conservation and relative homology with the human model. Thus, despite the complexity in the etiology of neurodegenerative disorders, a possible approach is a suitable behavioral test battery that allows the correct identification of the characteristic symptoms of the diseases that we study, and the proper definition of the parameters evaluated in our mouse models. In this context, research in both, basic and applied biology, require these models of human disease and, above all, its correct phenotyping to elucidate its underlying mechanisms and, consequently, generate appropriate targeted therapies. In this talk we will briefly review what are the most interesting murine models of neurodegeneration and what is the most appropriate behavioral approach, specifically addressed to assess the characteristics that modeling the human disease in mice, and focused in preserving animal welfare.

S27-2 Primate Models of Parkinson Disease: Why? How? And Refinement

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Parkinson disease is one of the most common and devastating human disorders. The disease is characterized by motor symptoms (poverty of movements, muscle rigidity and tremor) as well as by cognitive and emotional deficits. Nonhuman primates (NHP) treated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) neurotoxin reveal very similar clinical symptoms, response to pharmacological (for example, dopamine replacement therapy) and surgical (for example, deep brain stimulation) treatments of Parkinson disease as human patients. That is the reason why MPTP treated monkeys are commonly used in studies seeking for better understanding of the basal ganglia and for improved therapeutic methods for Parkinson disease. MPTP can be administrated to NHP either systemically or through carotid artery in acute or chronic ways. The systemic MPTP primate model offers the most similar model of the human conditions; however the severe clinical symptoms of the treated monkeys demand intensive care that should include frequent follow ups (general condition, weight loss and pressure ulcers), multiple nasogastric feeding sessions/day, isolated housing and frequent position changes. In my talk, I will review the anatomy and physiology of the basal ganglia and dopamine system, and why dopamine depletion in the basal ganglia is leading to the clinical symptoms of Parkinson disease. I will discuss the advantage and the disadvantages of the different primate MPTP models as well as their ethical use. I will give recommendations for minimizing the biohazard effects (to the experimentalists and the animal facility workers) of the MPTP toxin. Finally, I will present current refinement strategies for MPTP treated Parkinsonian monkeys.

S27-3 Care and Management of Animals Models of Neurodegenerative Disease

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The use of animal models to study neurodegenerative diseases (ND) can present scientists, veterinarians, and compliance personnel with challenging animal care and welfare concerns because animals with ND can manifest abnormalities in movement, behavior, learning, and memory. Several approaches can be used to improve the welfare of animals with ND including identification of humane endpoints, modification of husbandry procedures, and veterinary medical intervention. Through protocol review, the ethics committee or Institutional Animal Care and Use Committee (IACUC) can assure the humane care of animals with ND. The IACUC should inquire of the investigator whether the scientific aims can be accomplished through the use of animals with mild symptoms in the early stages of the disease. Scientists should be required to define specific endpoints that would limit the development of serious impairment in animals. In some cases, behavioral tests can be utilized to assess the degree of impairment and can assist in defining humane endpoints. Animal care personnel can modify husbandry procedures to accommodate movement abnormalities or muscle weakness if animals may have difficulty reaching the food or water. Group housing and environmental enrichment can improve animal wellbeing although it must be recognized that environmental enrichment can alter phenotypic expression in some models. Veterinary and nursing care should be provided if animals develop secondary conditions such a decubital sores, weight loss, or poor body condition. Some animals may exhibit changes in urination and defecation. With sufficient forethought, animal welfare can be preserved for most animals used in ND studies.

S28-1 Cross Company Data-Sharing in Pharmaceutical Development: The Good, the Could, and the Should

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The NC3Rs has an established reputation as an honest-broker for sharing data, knowledge and experience between companies and sectors. We work with the international pharmaceutical, chemical and consumer products industries and regulatory bodies to identify and share opportunities which benefit science, business and the 3Rs. Our data-sharing initiatives span a wide range of areas from the use of human tissue to replace animal use in safety studies to reducing the use of nonhuman primates in biologic development. We have learnt through experience that creating a trusted environment, working internationally and developing shared objectives are essential to success. There are multiple benefits to the NC3Rs approach such as the input of different expertise, generation of a larger evidence base to influence regulatory change and the acceleration of change into practice. The power of partnerships and what they can deliver will be demonstrated through case studies from the pharmaceutical and chemical industry.

S28-2 EEG Markers of Motor Activity in Wildtype and TASTPM Mice: A Multilaboratory Data-Sharing Experience in the Framework of Pharmacog Project

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The present study evaluated the spectral electroencephalographic (EEG) markers of motor activity in wild type (WT) mice and TASTTPM transgenic mice. To address this issue, EEG data were recorded from a monopolar parietal or bipolar frontoparietal electrode in 58 WT mice (5 females; range of age: 4.5 to 24 months) and in 15 TASTPM mice (4 females, 12-14 months) in four laboratories in the framework of the European project PharmaCog. Artifact-free EEG segments during the wake active state (gross movements, exploratory movements or locomotor activity) and the awake passive state (no sleep) were extracted on the basis of behavioral state of the mice during the EEG recordings. The artifact-free EEG segments for the active and the passive state were used as an input for EEG power density analysis. Results showed that in WT mice normalized EEG power density was higher in the passive compared to the active condition at 1-2 Hz, 2-4 Hz, and 4-6 Hz ($P < 0.05$). On the contrary, the normalized EEG power density was higher in the active compared to the passive condition at 6-8 Hz and 8-10 Hz ($P < 0.05$). Compared to WT mice, TASTPM mice were characterized by a reduced neural synchronization generating low-amplitude slow EEG oscillations at 2-6 Hz during the passive state and low-amplitude EEG oscillations at 8-10 Hz during the active state. These results suggest that WT and TASTTPM mice are characterized by peculiar EEG markers of motor activity and may be useful in preclinical drug research.

S29-1 Modeling the Host-Microbial Superorganism: Taking Experimental Animal Husbandry to the Next Level

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Humans and native animals coexist with an extremely diverse microbiota on body surfaces consisting of approximately 1000 taxonomic units. It is known that there is promiscuous exchange of metabolites between the host and the microbiota, and that the presence of commensal microbes in the intestine shapes the structure and function of almost every host organ system. We have a serious problem in modeling human disease reproducibly in that different vivaria housing experimental animals have different microbiota compositions in their colonies. These microbiotas are usually not monitored in detail over time, but when monitored we know that they tend to be somewhat unstable both between colonies in an animal house and within a single colony over time. Unfortunately, the defined gnotobiotic altered Schaedler flora (consisting of 8 defined fastidious bacteria), which is the current method of standardizing a microbiota, is so limited that it fails to model the metabolic embrace with the host adequately. We have developed medium-diversity defined microbiotas in gnotobiotic mice, and these will be discussed in relation to the technical issues of maintaining mice with standardized microbiota and the phenotypic differences with germ free or mice kept under current specific pathogen-free husbandry conditions

S29-2 A European Consortium for Gnotobiology

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Homeostasis between the intestinal microbiota and the host ensures vital functions of the organism, such as efficient energy and metabolite extraction from food, xenobiotic metabolism, defense against pathogenic microbes and maintenance of a robust epithelial barrier. Disruption of this homeostasis can lead to an array of severe illnesses with a major impact on public health. Inflammatory bowel disease (IBD) is only one example of many diseases that involve the symbiotic microbiota. For these reasons, mapping and understanding the interactions between the microbiota and the host is the basis of numerous large projects in Europe and beyond. Essential tools for this type of research are germfree (GF) and gnotoxenic (GX) animals. Several research centers have developed independent resources to generate GF and GX animal models in order to decipher the role of particular symbiotic microbial species during homeostasis or pathology. Ten European centers have expressed an interest to coordinate their efforts in Gnotobiology in order to improve exchange of expertise and resources, regarding in particular: (i) the coordinated production and distribution of GF and GX animals; (ii) the validation of GF and GX status; (iii) the development of technology (that is, housing equipment); (iv) the improvement and harmonization of shipping vehicles.

S29-3 Hygienic Monitoring of Germ-Free and Gnotobiotic Animals

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Germfree and gnotobiotic animals are presently used only in few institutions. It has to be expected that such animals as well as gnotobiotic techniques will get increasingly important in the future especially in studies that aim at assessing the contribution of the microbiome to specific phenotypes. Such animals need specific housing conditions and absolutely sterile handling and supply. The risk of introducing microorganisms is low but it does occur. Contamination will most likely be caused by environmental organisms like certain bacteria or molds. Sterility of the housing isolator will be checked prior to the introduction of animals. After entry of animals, the status needs to be verified on a regular basis by frequent inspection of the isolator and the animals including olfactory examination. Laboratory diagnostic procedures such as microscopic examination of intestinal contents need to be performed. Most importantly, aerobic and anaerobic culture at different incubation temperatures is necessary to detect or exclude bacteria and fungi. Swabs from internal surfaces of the isolator may be tested at a higher frequency but also testing of complete animals for bacterial and fungal contamination or parasites is necessary on a regular basis. Testing for viruses is possible by serological methods. Testing for specific agents (for example, Helicobacter sp., Pneumocystis sp., and certain viruses) who may not be detected by traditional methods can be performed by PCR. Some laboratories already use PCR also to detect or exclude bacteria in general.

S29-4 A Multihit Model: The IL10-Deficient Mouse Model for Inflammatory Bowel Disease

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Inflammatory bowel disease (IBD) is a chronic relapsing disorder characterized by an excessive immune response that is driven by the complex interaction of genetic and environmental factors. We aimed at elucidating the interaction of microbiota and genetic factors in the Interleukin-10 (IL10)-deficient mouse model of IBD.IL10-deficient mice showed considerable differences in disease expression in colitogenic environments depending on the genetic background, mainly driven by a genetic contributor defined as *Cdcs1*. To identify geneXmicrobial interactions, susceptible and resistant IL10-deficient strains as well as *Cdcs1*-congenic strains were sanitized in non-colitogenic SPF environments and derived germ-free. Inflammation was induced by DSS treatment, *Helicobacter hepaticus* or Murine Norovirus (MNV) infection. Interestingly, original susceptibilities were only partly reflected after sanitation. For example, after *Helicobacter* infection, resistant B6-IL10-deficient mice demonstrated a milder inflammation in the cecum than their susceptible C3-IL10-deficient counterpart but more severe lesions in the colon. *Cdcs1*-congenic strains remained susceptible. *Helicobacter* did not induce inflammation under germ-free conditions regardless of the background. The discrepancy to the original phenotype was

also found after using DSS for colitis induction. Infection with MNV triggered a mild mucosal inflammation in the IL10-deficient host, which was modified by genetics and absent under germ-free conditions, therefore microbiota-driven. In addition, mouse strain specific shifts in the microflora were determined during *Helicobacter*-induced inflammation. In summary, we can outline the IL10-deficient mouse as a multi-hit IBD model: A colitogenic environmental trigger initiates the inflammatory process, strain specific genetic factors influence the inflammatory response and, additionally, the microflora modifies these strain-specific or genetic susceptibilities.

S29-5 Gut Commensal Microbiota and TLR Expression Variations in Rats Depending on Commercial Origin

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Gut microbiota is involved in gastrointestinal inflammatory diseases. It is known that genetic background and environment influence microbiota composition, leading to variations in experimental models. We characterized gut microbiota in a rat strain (SD) from different vendors (A, B, C and D; $n = 8$, both sexes) in 2010 and 2011. Luminal and adhered ceco-colonic microbiota were assessed using fluorescent in situ hybridization. Toll-like receptors (TLR) 2, 3, 4, 5, 7, 9 and mucin (MUC2) expression were RT-qPCR quantified in colonic tissues. In general, neither sex, nor temporal differences in luminal microbiota were found. However, significant differences in composition amongst rat origin were detected. For instance, group B showed significantly higher counts for *Lactobacillus-Enterococcus* spp., *Bifidobacterium* spp. and Enterobacteria populations, and group A showed significantly higher counts for *Bacteroides* spp. All groups displayed mucosal attachment of *Clostridium* spp. (Within 60-90%). In most cases, bacteria found at higher numbers at the lumen where the same mucosa-attached. Group A exhibited adhered *Bacteroides* spp. (12%), whereas *Bifidobacterium* spp. and *Lactobacillus-Enterococcus* spp. where attached in group B. TLR 2, 5 and 7 expression was significantly lower in group B compared to the rest ($P < 0.05$, $P < 0.001$, $P < 0.01$). TLR expression was correlated in some cases with the counts of luminal bacteria, for example, TLR 2 and 4 showed negative correlation with *Lactobacillus* spp. and *Bifidobacterium* spp. counts ($P < 0.05$, $r^2 = 0.12$-0.17, respectively). These observations confirm that microbial and immune differences in the same rat strain exist depending on the vendor and might entail different susceptibilities to inflammation and onset of gastrointestinal disease models.

S29-6 The Phenomenon of Bacterial Translocation from the Gut in Experimental Fungal Infection Neutropenic Animal Models

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Experimental fungal infections in rodents are usually studying challenge by the pathogen after transient neutropenia. In case transient neutropenia is harmful to the gut mucosa bacterial translocation from the gut may happen. To investigate this hypothesis $5x10^5$ cfu/ml of one *Candida parapsilosis* isolate was intravenously infused in 28 rats rendered neutropenic after a course of cyclophosphamide. Survival was recorded and yeast and bacterial outgrowth of liver, spleen, lung, and kidney was measured after sacrifice at serial time intervals. Animals rendered neutropenic with two administered intraperitoneal dose of cyclophosphasmide; the first of 150 mg/kg 96 hours and the second 100 mg/kg 48 hours before challenge. Achievement of neutropenia i.e less than 1000 neutrophils/mm^3 was confirmed in four rats by blood sampling 48 hours after the second dose of cyclophosphamide. Mean respective log 10 of enterobacteria in the liver at 24, 48, and 72 hours were 4.67, 2.33 and 4.36; in the spleen 4.03, 2.25 and 4.20; in the lung 4.19, 2.21, and 2.87; and in the right kidney 4.42, 2.10 and 3.68. At the same time intervals respective yeast outgrowth in the liver was 3.84, 3.98 and 3.80, in the spleen 3.85, 4.91 and 4.78; in the lung 4.54, 5.82 and 4.82; and in the right kidney 4.27, 4.66 and 4.79 It is concluded that the described model which is broadly used in the literature is characterized by bacterial translocation from the gut. This should always bear in mind when this model is used to assess the efficacy of newer antifungals.

S30-1 An Indirect Calorimeter for 200 Euros?

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As the human obesity epidemic has progressed, interest in using rodent models of obesity to study the mechanisms behind weight gain has increased. Obesity occurs as a result of chronic positive energy balance. Energy balance is defined as the difference between energy intake and energy expenditure. A critical question when assessing any rodent model of obesity is whether its increased body weight is caused by increased energy intake or decreased energy expenditure. Assessment of energy intake is usually performed by weighing food intake, whereas assessment of energy expenditure is assessed using a variety of direct, or more commonly indirect calorimetric techniques. While calorimetry gives straightforward assessment of energy expenditure, most commercial murine calorimeters cost in excess of 100, 000 Euros, limiting their general availability. This talk focuses on how utilising only a set of accurate weighing scales to assess both food intake and body weight changes, it is possible to determine if an animal model exhibits changes in energy expenditure with a high degree of accuracy. Furthermore, the talk will discuss the differences between energy balance and metabolic rate and methods to normalise energy expenditure for body weight.

S30-2 Rising to the Challenge of EU/2010/63: Reducing Severity of Animal Studies of Diabetes

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The implementation of refinement in animal experiments is currently underutilized. The enforcing of more stringent regulations is seen as a driver for the improvement of animal welfare. However, recent data have shown that reported regulatory compliance is often not reflected in the choice of methods and that refinement measures are insufficiently implemented. While refinement measures are often regarded as disrupters of standardization, there is little information on their actual impact on reliability or reproducibility of animal experiments. Stress, however, has been shown to influence pathology in several animal models of human diseases, including in murine models of diabetes. The influence of stress in such models has been shown to vary according with type of stressor and intensity, but also with gender and type of animal model. The potential –and unpredictable– effect of environmentally-induced stress in animal models of diabetes makes it the more important to minimize its impact as a source of undesirable variability. Also, with the new 2010/63/EU Directive, protocols submitted for approval must include a prospective assessment of animal pain, suffering and distress as well as indication of how suffering will be minimized. Taking refinement of procedures as basic ethical, scientific and regulatory requirements, this presentation will focus on possible refinements for animal studies on diabetes. The possible impact of refinement on variability and reproducibility will be discussed, along with its potential to improve both animal welfare and the quality of research in these studies.

S30-3 Rat Models of Obesity Used for Insulin Resistance Studies: A

Comparison from the User's Perspective

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The association between obesity, insulin resistance and diabetes mellitus has been well established, and has relied substantially on animal models of obesity, namely rat models. Different rat models, with distinct obesity genesis, present different metabolic outcomes. The aim of this presentation is to compare two rat models of obesity in terms of fasting and postprandial glucose metabolism. The models analyzed were the high-fat diet (HFD) rat (Sprague–Dawley rats on HFD for 4 weeks) and the obese Zucker rat (OZR); their controls were normal chow-fed Sprague–Dawley (STD) and Lean Zucker (LZR) rats, respectively. Animals were tested at 9 weeks, under anesthesia, either in the fasted or postprandial state. Body weight, blood pressure, glycemia, insulinemia and insulin sensitivity assessments were made. Both obese groups were significantly heavier than their controls. Fasting glycemia was similar between obese and non-obese animals; however, postprandial glycemia was higher in HFD than in STD. Plasma insulin levels were ~17 times higher in the OZR than in LZR, whereas HFD presented an insulinemia ~2 times that of STD, suggesting that normoglycemia in OZR is achieved mainly through pancreatic hypersecretion. Both obese models were insulin resistant when compared with non-obese controls; however, in the HFD, the difference was in the postprandial state only, whereas in the OZR both fasting and postprandial insulin sensitivity were impaired. In conclusion, different rat models of obesity present different patterns of insulin resistance and, concomitantly, different mechanisms of glucose metabolism impairment. The selection of which model to use should have this aspect in consideration.

S30-4 Correlation between Metabolic Syndrome and Renal Injury in Porcine Model

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Metabolic syndrome is a global public health problem, with its importance deriving from its high prevalence and foreseeable increasing tendency in the near future. The progressive impairment of renal function is one of the largest problems in patients presenting metabolic syndrome (MS).The purpose of the present study is to observe the relationship between the analytical lipid profile and the degree of renal histologic lesion in porcine animal model. We used 13 male Gottingen Minipigs, for the creation of a metabolic syndrome animal model nourished with hypercaloric feed during 9 months. Renal analytic parameters (urea, creatinine and total protein), and lipid profile (HDL, LDL and triglycerides) were obtained at basal levels (T0) and after 9 months of a saturated fat rich diet (T9). Also, renal samples were taken for anatomopathologic study. Glomerular degeneration and fibrosis together with glycogenic nephrosis due to hyaline deposition relate to an increase in biochemical parameters: Urea (T0: 21,88 mg/dl compared with T9: 25,82 mg/dl), creatinine (T0: 1,63 mg/dl compared with T9: 1,95 mg/dl), total protein (T0: 8,15 g/dl compared with T9: 7,38 g/dl), HDL (T0: 21,71 mg/dl compared with T9: 46.75 mg/dl), LDL (T0 19,64 mg/dl compared with T9: 31,87 mg/dl) and triglycerides (T0: 33,42 mg/dl compared with T9: 50 mg/dl). Diet-induced hypercholesterolemia produces mild glomerular damage, reflected on biochemical analysis and at histologic level. Thereby, this study provides a useful tool for the diagnosis and treatment of progressive renal impairment triggered by lipid metabolism.

S30-5 The Influence of Oral LPS on Development of Glucose Intolerance in High-Fat Fed Mice

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The gut microbiota (GM) has been suggested to influence development of type 2 diabetes (T2DM) in many different ways, one being immune stimulation. Various studies have suggested lipopolysaccharides (LPS) from gram-negative bacteria as a factor in development of T2DM, as it binds TLR receptors, initiating inflammatory responses, which contribute to the low grade inflammation characterizing T2DM. However, these studies have demonstrated an influence of LPS on glucose intolerance (GI) only after intravenous or subcutaneous administration. We therefore tested whether LPS given orally influence the development of GI and other parameters related to T2DM. We hypothesized, that C57BL/6 mice given a high-fat diet supplemented with LPS would express a higher level of GI, inflammation and other T2DM parameters than control mice given a standard high-fat diet. By performing an oral Glucose Tolerance Test we demonstrated, that mice receiving an LPS containing diet had a higher level of GI than the control after 12 and 16 weeks on the diet. Further investigations concerning other T2DM related parameters (adiposity, inflammation, HbA1c) and how LPS given orally influences the immune system locally in the gut and systemically and its correlation to GI is ongoing. Our results indicate that it may be possible to manipulate disease development of T2DM through oral administered bacterial components.

S31-1 Severity Assessment under Directive 2010/63/EU

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Directive 2010/63/EU on the protection of animals used for scientific purposes requires that a PROSPECTIVE assessment is made on the severity of each procedure in a Project and that a severity classification is assigned, which may be either "non-recovery", "mild", "moderate" or "severe". Annex VIII provides guidance on the factors to be taken into account in the consideration of prospective severity and provides some examples of procedures in each severity category. These do not illustrate how refinements can reduce severity. For statistical reporting, the ACTUAL severity of the pain, suffering, distress or lasting harm experienced by the animal must be reported. In addition, the actual severity of any previous procedures will be a key consideration in determining whether or not an animal can be reused in further procedures. These measures provide opportunities to improve the quality of science and welfare through prospective review of project proposals promote effective monitoring during the procedures and, by inclusion of the actual suffering experienced by the animal, should provide greater transparency and understanding of the impact of scientific procedures on animal welfare. The Commission established an Expert Working Group (EWG) for the assessment of severity of procedures to facilitate the implementation of Directive. The first meeting focused on severity in genetically altered animals, the second on developing a general framework for assessing the actual severity experienced by animals in procedures. The outcomes were endorsed by the National Contact Points of the Member States in July 2012.The presentation will summarize the outcomes from the EWG.

S31-2 Report of FELASA/ESLAV Working Group on Severity: Approach of the WG

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In view of the requirements in the new Directive 2010/63/EU for Se-

verity Classification and Assessment, a Working Group was established by FELASA, subsequently enlarged to include input from ECLAM and ESLAV, to provide guidance to those involved in the care and use of animals on severity assessment and to prepare some illustrative worked examples. Although Annex VIII provided some guidance on prospective classification, many within the scientific community raised concerns that some of the examples provided therein contained insufficient information to satisfactorily explain the rationale for the severity classification chosen and that no examples were provided for some common areas of research, such as pain and arthritis models. Without additional explanation it was considered that there were likely to be considerable differences in the assignation of severity, which would ultimately give misleading information on animal use, and, perhaps of greater concern, result in inappropriate re-use of animals. The WG has developed a framework for the assessment of severity and developed a range of models covering many areas of research, from models of high severity such as stroke and arthritis to mild behavioral models. The presentation will explain how the WG developed their approach to severity assessment, including the factors to be taken into consideration, the importance of effective monitoring and recording, and the various methods which can be used to assist in assignment of severity.

S31-3 Severity Assessment of Mouse Models Used in Oncology Studies

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A number of animal models are currently used to improve understanding of the mechanisms involved in tumor development and in the development of new treatment strategies. The severity of the effects on the animals will be dependent on the models and the purpose of the study. For example, the maintenance of tumor cell lines should not have a significant impact on welfare provided that good practice is observed throughout, including appropriate animal monitoring and the adoption of early humane end-points. In contrast, studies to assess novel treatments in metastatic models are likely to have more significant welfare concerns due to multiple tumor development and the likely adverse effects of cytotoxic drugs. Two examples developed by the WG will be used to illustrate oncology animal models of differing severity.

S31-4 Severity Assessment of a Rat Stroke Model

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Stroke is a leading cause of sudden death and serious, long-term disability in developed countries that, to this date, remains an unmet medical need. Animal models of stroke, by their very nature, represent a challenge from the perspective of animal welfare, and every effort must be made to ensure the minimum possible animal cost together with the maximum benefit in scientific terms. A commonly used stroke model in rats (Middle Cerebral Artery Occlusion, MCAO) will be used as an example to illustrate the severity assessment process as requested by the new Directive 2010/63/EU. To do so, prospective and retrospective severity assessment will be developed, using the structured approach adopted by the FELASA/ ESLAV/ ECLAM Working Group on Severity Classification.

S31-5 Severity Assessment: A Useful Refinement Tool in Regulatory Toxicology Studies

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Regulatory requirements in toxicology aim to protect human and animal health, and the environment. The field of regulatory toxicology may range from pharmaceutical drug discovery to risk assessment of (agro-)chemicals after human or ecological exposure. Before animal studies required by testing guidelines are performed, all data (literature, physic-chemical, in silico, in vitro, possible previous in vivo studies) should be available to perform a weight of evidence analysis to assess potential toxicity of the chemical and to determine the justification of (further) animal testing. Typical regulatory toxicology animal models will be used as examples to show the severity assessment process as required by the European Directive 2010/63. Procedures, their possible adverse effects, methodology and interventions to minimize severity including consideration of humane endpoints in these models will be discussed. This overview per specific toxicology animal model will, together with consistent performance of well-designed protocols/procedures by competent personnel, assist in the overall severity assessment process of project/procedure planning, monitoring, corrective actions, retrospective analysis and communication/feedback to personnel involved. Prospective and retrospective severity assessment of each project/procedure may thus be regarded as a useful refinement tool to, where possible, improve study designs and procedures at institutional as well as regulatory authority level to ultimately benefit animals and science. The regulatory toxicology models are some of many examples adopted by the FELASA/ESLAV/ECLAM Working Group on Severity Classification.

S31-6 Severity Assessment of a Rat Arthritis Model

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Animal models of arthritis are used to study the pathogenesis of the disease and to evaluate potential anti-arthritic drugs for clinical use. The model presented in this example is Type II Collagen arthritis in rats. This model produces clinical signs similar to those seen in human rheumatoid arthritis as a consequence of cartilage destruction, bone resorption, moderate to marked synovitis and periarticular inflammation. The model can cause severe suffering. The presentation will illustrate how the prospective severity assessment of the model can be carried out based on the methodology of the FELASA/ESLAV/ECLAM Working Group. It will also be discussed what experimental conditions should be fulfilled and how appropriate refinement tools can be applied to keep the model below the upper limit of severity as defined by the 2010/63 Directive. Examples of actual severity assessments will be given highlighting that the actual animal suffering can still be differentiated even if the overall severity category of the model remain severe.

S31-7 Severity Assessment in GMOs

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On 22/09/2010 the new EU Directive 2010/63/EU became applicable. One essential claim of the directive is the authorization of projects aimed to generate and to maintain genetically altered lines and strains when a harmful phenotype can be expected or is already apparent. A harmful phenotype is delineated as "a consequence of the genetic alteration pain, suffering, distress or lasting harm equivalent to, or higher than, that caused by the introduction of a needle in accordance with good veterinary practice". Two expert working groups were established by the EU. They should develop a common format for statistical reporting and for the assessment of severity of procedures and to define the requirements of the new EU Directive precisely to facilitate the member states to transfer the directive into national law. The resulting working paper supposes procedures and criteria for an appropriate assessment of genetically altered animals. Here we report the attempt to assess the

phenotype of different genetically modified strains using the supposed criteria and additional analysis such as MRI. Our findings show how difficult it is to assess and to predict the severity of a phenotype when (a) an altered gene is expressed onto different genetic backgrounds and (b) none of the supposed criteria are indicating a harmful phenotype although an organ is clearly affected by the genetic alteration.

S32-1 Current Status of Nanopharmaceuticals

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Despite the recent introduction of the "nanomedicine" term in scientific literature, the origin of nanocarriers for drug delivery, dates from the early 60's. These original ideas have already led to the commercialization of a significant number of nanopharmaceuticals. However, the full potential of nanomedicine is still to come. This potential is promising because over the last decades we have initiated a non-return pathway, which is the adoption of transdisciplinary and translational approaches to the design and development of new nanopharmaceuticals. In particular, the advances made over the last decade have been largely related to the progress made in the biomedical field (improved knowledge of disease mechanisms, identification of new targets and new bioactive compounds, etc.) and the nanomaterials field (new biomaterials and nanotechnologies). The nanopharmaceutical technology area has also contributed making it feasible the nanoencapsulation and controlled delivery of complex molecules, as well as defining ways to scale-up the production of nanomedicines. Nevertheless, an area that requires special attention is the one related to the analytical techniques and models for investigating the mechanism of action, PK and toxicology issues of nanopharmaceuticals. Our group has designed novel nanostructured materials intended to transport drugs and antigens across biologic barriers and to deliver them to the target tissue. During my presentation I would like to focus on the techniques and in vitro-in vivo models that we have explored until now to assess the behavior of the nanopharmaceuticals developed in our group. More information about these studies can be found at: http://webspersoais.usc.es/mariaj.alonso

S32-2 What Are Cells Telling Us about Nanomaterial Safety?

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Nanomaterials hold great promise for a variety of applications, due to their outstanding properties. The production of certain materials achieved industrial scale. Therefore the exposure risk to humans has also increased substantially and induced concerns about their potential adverse health effects, predominantly because of to their size, shape and chemistry. The current approach of regulatory toxicology is to test biologic effects of any xenobiotics such as new chemicals, drugs or nanomaterials mainly based on animal testing - a strategy which has not changed in the last 40 years. Therefore new concepts for more efficient, cheaper and evidence based test strategies were proposed. In most of these concepts a shift from phenomenological analyses in animals studies towards mechanistically-based assays using cells and cell lines are envisioned. In order to initiate the reduction, refinement and replacement of time and cost intensive in vivo experimental approach, many advanced and complex human cell models have been developed during the last decades. However these activities are still in their infancy and the clinical reliability of such cell culture models is not always given. The aim of this paper is to discuss the current state of 3D co-culture models representing different human organs/tissue barriers and their contribution in nanosafety research.

S32-3 Nanoparticles as a Valuable Tool for In Vivo Toxicology

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The use of nanoparticles, defined here as any material with a measurable dimension in the nm scale (between 1-100nm) in pharmacology, medicine, diagnostics and other life science disciplines is the subject much research work and debate. The opportunities associated with novel physicochemical properties offers special challenges in the regulatory acceptance of nanomedicine. The purpose of this presentation is to highlight some of the areas where coin is somewhat turned, and where we ask ourselves; how are nanoparticles useful as a tool in toxicology? How can nanoparticles speed up long process such as drug discovery, toxicology assessment of lead candidates or offer alternative methods of drug administration? We will review examples of the use of silica-based nanoparticles for toxicology assessment of poorly soluble lead candidates with poor bioavailability in an in vivo context. The presentation will highlight how novel nanoparticles based on quantumn dots can be used as contrast agents to follow the uptake and localization of active drug compounds in vivo, and finally, we will describe how nanoparticles are enabling previously difficult translational research to proceed towards clinical trials.

S32-4 Early Detection of Hepatotoxicity in Drug Development Process

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Hepatotoxicity is a major reason for drug non-approvals and withdrawals. This study aimed to develop and validate a practical, reproducible, in vitro multiparametric cell-based protocol to assess those drugs that are potentially hepatotoxic to humans and to suggest their mechanisms of action. The assay was applied to HepG2 human cell line cultured in 96-well plates and exposed to 78 different compounds. After treatments, cells were simultaneously loaded with five fluorescent dyes showing optical compatibility and were then analysed with the High-Content Screening Station Scan. Use of the HepG2 cell line to assess hepatotoxicity induced by bioactivable compounds is hampered by their low cytochrome P450 expression. To overcome this limitation, we further developed upgraded HepG2 cells expressing functional levels of the major P450 enzymes involved in the oxidative metabolism of drugs in human liver (ADV-HepG2. The strategy is based on combining ADV-HepG2 cells and high-content screening technology to simultaneously measure multiple parameters indicative of cell injury. ADV-HepG2 and HepG2 cells were exposed to of 12 bioactivable and 3 non-bioactivable compounds. This strategy appears to identify early and late events in the hepatotoxic process, and also suggest the mechanism(s) implicated in the toxicity of compounds to thereby classify them according to their degree of injury (no injury, low, moderate and high injury). Overall, our results indicate that this assay may be a suitable new in vitro approach for early screening of compounds to identify bioactivable hepatotoxins and the mechanism(s) involved in their toxicity.

S32-5 The International Consortium for Innovation and Quality in Pharmaceutical Development (IQ) in 3Rs Leadership Group: The Pharmaceutical Industry's Promotion of Alternatives

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In January 2012, the 3Rs Leadership Group (LG) of the International

Consortium for Innovation and Quality in Pharmaceutical Development (IQ) was established. The 3Rs LG is made up of senior veterinarians, biomedical scientists and 3Rs specialists from IQ member pharmaceutical/biotechnology companies. The mission of the 3Rs LG is to promote sharing and integration of high quality scientific practices to advance the Replacement, Reduction, and Refinement of animals used in the discovery and development of new medicines, vaccines, medical devices and health care products for humans and animals. The first year was dedicated to creating a variety of Working Groups to promote 3Rs in a range of areas and initiating several 3Rs projects. One of the main goals of the 3Rs LG is to gather benchmarking information about alternatives from across the industry to highlight strengths and identify gaps. Another goal is to facilitate communication and education about 3Rs advances in a more systematic manner across the biomedical research community via WebEx conferences, journal articles and seminars at international meetings. A fourth goal is to develop industry consensus on positions and alternatives to advance science, welfare, and innovation on 3Rs issues with external stakeholders (legislators, regulators, NGOs, CROs, academia). This presentation will provide a more detailed overview of the 3Rs LG and its various WGs and current 3Rs initiatives to further our collaborative reach and progress our goals of communicating 3Rs advances globally.

S33-1 Novel Animal Models for Blood and Immune Disorders and Basics of Stem Cell Biology

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A strict balance between self-renewal and differentiation of hematopoietic stem cells (HSCs) is required in order to maintain homeostasis, as well as to efficiently respond to injury and infections. We and others have shown an important role for Wnt signaling in this process. Mouse models have been crucial to understand the basics of stem cells and have led the foundation for human stem cell therapy. Studies with experimental animal models have produced contradictory findings regarding the importance of Wnt signals for normal hematopoiesis. Using the targeted approach of the APC tumor suppressor gene, we showed an essential and dosage-dependent regulation of hematopoiesis by Wnt signals. For study of human HSCs in vivo by transplantation into immune deficient mice is used. We have used both $Rag2^{-/-}\gamma_c^{-/-}$ and NSG for creating humanized mice. Newborn mice are generally used for the transplantation of human HSC. There are several disadvantages using newborn mice, such as rejection by the mother after handling, more difficult routes of transplantation compared to the tail vein and the need for timed pregnancies for which larger breedings are needed. Using NSG mice, high (>80%) chimerism and presence of all human blood cell types, including T-lymphocytes was observed. NSG mice can be immunized with T cell dependent antigens, resulting in production of human antibodies, indicating collaboration of human T, B and DC in these mice. I will illustrate use of these mice for elucidating the mechanism of T-ALL oncogenes, preclinical gene therapy experiments and discovery of a novel type of human SCID.

S33-2 Replacement of the In Vivo Assay "Teratoma" with a New In Vitro Bioinformatic Assay for Testing Pluripotency of Stem Cells: Development and Validation

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The startling discovery of 2007 that human somatic cells can be reprogramd towards a pluripotent stem cell-like state (induced pluripotent stem cells, iPSC) has opened a new era for the understanding and treatment of human genetic diseases. Since then hundreds if not thousands of iPSC lines have been generated and the increase is expected to be exponential. The reason for this is that reprogramming is a relatively simple method on the one hand and that iPSC derivatives could be used in drug and toxicology testing as well as for customized cell replacement therapies in the future on the other hand. A recent EU call under an Innovative Medicine Initiative program co-financed by the five largest pharmaceutical companies requested the generation of 10,000 disease lines, in just one project. Similar as for human embryonic stem cells the gold standard method for determining whether an iPSC line is truly pluripotent is to inject them into immunodeficient mice where they form tumors (teratomas), which contain derivatives of all three germ layers. Each iPSC line needs to be tested for pluripotency. In addition to ethical issues and animal welfare concerns the assay is primarily qualitative, difficult to standardize, resource intensive, and time-consuming. Since the number of mice used for teratoma assays is expected to rise dramatically there is an urgent need for the development of an alternative *in vitro* assay. Our aim is to replace the teratoma assay with a data driven in vitro test based on transcriptional profiling by microarray.

S33-3 The Porcine Myocardial Infarction Model: Surgical Approach and Tissue Engineering

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Myocardial infarction (MI) is the leading cause of mortality and morbidity in developed and developing countries. MI caused by coronary artery occlusion elicits myocardial necrosis resulting in structural and functional adaptations flowing to heart failure. Current therapies such as clot lysing and stenotic artery revascularization are partially effective and many patients receive medical assistance too late: "time is myocardium". Given these circumstances, research in new MI treatments is needed to improve quality of life and life expectancy. In this context, cardiac tissue engineering is emerging as a new experimental approach based on the use of cells, scaffolds, growth, differentiation and proangiogenic factors, to finally regenerate the infarcted myocardium. Knowing that coronary artery pattern in swine is remarkably similar to that of humans, pig is one of the most advisable animal models in MI studies. The aim of this work is to evaluate new approaches in cardiac tissue engineering to diminish the adverse effects caused by MI in swine. The first procedure presented is this work is a new surgical technique based on the transposition of an autologous pericardial-derived vascular adipose flap onto the ischemic myocardial surface. The second approach consists in a new on-line bioactive smart patch with GFP-adipose tissue derived progenitor cells after MI in swine. In both procedures, left ventricular (LV) ejection fraction and LV end-diastolic and end-systolic volumes were assessed using magnetic resonance imaging. Infarct size, histologic changes, collagen volume fraction, and vascular density were also evaluated on postmortem sections.

S33-4 Murine Model of Myocardial Infarction: Advantages and Pitfalls for Cardiac Regeneration

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Myocardial infarction produces severe ischemic cardiomyopathies where the myocardium is deeply affected being its complete recovery still an unresolved concern. Therefore, new therapeutic approaches to treat these patients are highly required. For this purpose, a surgical model that mimics human myocardial infarction in mouse has progressed as an important tool to find new treatments for cardiac regeneration. Here we showed how these therapeutic approaches have evolved from cell therapy to advanced tissue engineering. Despite progressive improvements achieved in the past years, the best cell type and delivery strategy are not well established. We described advantages and

pitfalls from three strategies for cell delivery into the murine model of myocardial infarction: intramyocardial cell injection, implantation of a cell loaded fibrin patch, and an engineered bioimplant (combination of chemically designed scaffold, peptide hydrogel, and cells). Dual labeling noninvasive bioluminescence imaging is also shown as an original tool for in vivo monitoring of cardiac-specific markers and cell survival after implantation.

S34-1 Animal Models of Cardiovascular Disease for Research and Training

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Cardiovascular diseases are a major cause of death and disability in developed countries. Large animal models are essential to assess safety and efficacy of new procedures, at the same time providing a platform for training specialists. For research purposes, sometimes the native models can be sufficient, as when studying the biologic reaction to implanted prostheses, or when handling tests are needed prior to clinical use of devices, such as catheters. Some disease models sometimes used include surgical creation of abdominal aortic aneurysms using a peritoneal patch to test endovascular exclusion devices, carotid aneurysms creation using a jugular vein pouch to evaluate embolization systems such as bioactive coils, myocardial infarction induction obtained by coronary artery occlusions of different location and duration, a versatile model that allows us to study varied regenerative approaches or electrophysiological procedures, etc. Similarly, in terms of training, it is generally enough to use the native models, in which many endovascular skills can be acquired or perfected. Nowadays it's important to consider the role of virtual reality simulators, which can enhance the learning experience while reducing the amount of animals needed. Sometimes, simple surgical models, such as vascular stenosis or carotid aneurysms can be created to train in their therapy. Rotational atheterectomy for coronary plaque removal can easily be modeled by deploying a femoral stent covering the origin of the deep femoral artery, and then crossing through with the system. Electrophysiological mapping and ablation can be learned in a model of healed reperfused myocardial infarction.

S34-2 Cardiovascular Imaging in Large Animal Models

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Many of the large animal models of cardiovascular disease are amenable to imaging techniques used in human medicine. Ultrasonic imaging can be used for vascular access or percutaneous punctures in dogs, cats, and pigs using pediatric or adult devices. Smaller animals, such as rabbits, may require Seldinger techniques for vascular access, but angiography can then be performed on standard clinical angiographic systems. Echocardiography can be performed to assess cardiac function in awake dogs and cats, but usually requires sedation or anesthesia in less tractable animals, for example, pigs and rabbits. Clinical human or veterinary ultrasonography equipment can be used with the appropriate probes. Magnetic resonance imaging offers the ability to assess myocardial function, perfusion, and anatomy without ionizing radiation using standard clinical 1.0-3.0T scanners but requires general anesthesia. Computed tomography can be performed more rapidly, but provides less anatomic detail and care must be taken to avoid excess radiation exposure with serial imaging. Positron emission tomography (PET) and Single photon emission tomography (SPECT) can be performed in dedicated head imaging or whole body systems, but the spatial resolution is limited compared to imaging murine species in microPET and microSPECT systems. However, the advantage of adapting standard adult or pediatric imaging protocols that can be readily translated to patients as well as new techniques is appealing. Moreover, the first develop imaging techniques in large animals and then perform clinical veterinary trials in client-owned pets with naturally occurring disease is attractive as well.

S34-3 Cardiovascular Research: An Overview of Minipigs and Pigs as Animal Models

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Pigs have been used in cardiovascular research for decades, even centuries when including the works of Versalius and Galen, and much has been learned about the human situation from this species. However, it was not until the 1960s and 70s that pigs and minipigs rose to prominence as animal models used in cardiovascular research - a position they have maintained since. This presentation aims to give the audience an overview of cardiovacular models in pig and minipig used in biomedical research research today. It will include fundamental research into cardiovascular disease as well as cardiovascular research relevant in the development of new medicines.

S35-1 Genetically Altered Models: Do They Translate? An Introduction

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Genetic modification has come a long way from the constitutive modification to the introduction of the controlled induced modification technology; from the single gene mutants to the combination of a variable number of modified genes in one animal. More advanced technologies and breeding schemes are introduced while our knowledge about the genome and the phenome is increasing every day. With all these instruments in our toolbox one would expect to create the perfect animal models for various aspects of different diseases in humans. However, not seldom are we confronted with results from genetically altered animal models that do not easily translate into the apparent similar affect in humans even when the initial evaluation was that of close resemblance. What are unknowns and which sources of variability are playing tricks on us? These are among the topics that are up for discussion in this session.

S35-2 Both Coding and Noncoding Genomic DNA Sequences Contribute to Define the Genetic Diversity and the Phenotype in Mice

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The publication of the human and mouse genomes, reported more than 10 years ago, served to illustrate the extraordinary similarities existing between human genes and their murine counterparts, which is the basis of the use of mice as useful animal models of human diseases. Both in the number of genes we, and the mice, have (about 20,000-25,000, depending on how we define and what we consider a gene) and also regarding the functions represented (99% of mouse genes have their homologous loci in the human genome) both genomes are highly similar. Actually, more than 90% of the mouse genome can be aligned with homologous segments in the human genome. However, the majority of these DNA sequences do not correspond to coding regions, to exons, known to represent about 2% of the genome, where, historically, most mutations resulting in altered phenotypes have been normally described. What do we (and the mice) have in the remaining 98% of our genomes? The best systematic response to this question came last September 2012, when the results of the project ENCODE were released. They concluded that over 80% of the human (and, likewise, the mouse) genome carry some function, "serves some purpose, biochemi-

cally speaking". Therefore, most of our (and the mouse) genomes carry many non-coding, intergenic DNA elements that shape and contribute to define precisely the function of genes they regulate, they contain the "instructions" the genes need to follow in order to function correctly. Therefore, alterations occurring in these instructions will also impact on gene function, and, hence, influence the phenotype, in humans and mice.

S35-3 More Knowledge with Fewer Animals in Less Time

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The mouse has proven to be an excellent model to identify genetic and environmental factors important in cancer development. Modeling complex diseases such as cancer in mice requires genetically engineered mouse models (GEMMs) carrying multiple defined genomic alterations. Introducing such modifications by conventional breeding is inefficient, time consuming and requires large number of mice. Recent advances in embryonic stem cell (ESC) technology permit efficient derivation of ESC lines from established GEMMs. These GEMM-ESCs are of such high quality that they can be used for production of experimental cohorts of high-grade chimeras carrying all desired genetic modifications that develop identical tumors with similar incidence and latency as compared to the original GEMM. This technology permits the creation of an archive of (compound) mutant GEMM-ESC lines, rather that live mice, thereby efficiently reducing the number of animals needed for breeding and maintenance of GEMM lines. The cumulative GEMM-ESC recourse not only allows investigators to quickly generate mice with these modifications, it also serves as a starting point for introducing additional modifications, for instance by controlled integration of a transgene, thus permitting the swift generation of mice with complex genotypes tailored to the needs of cancer research. Here, we present the feasibility and process efficiency of the GEMM-ESC pipeline.

S35-4 Managing Genetic Drift in Mice and Rats Models Colonies

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Even using co-isogenic or congenic animals, as the breeding scheme of animals leads to change the colony in a “close colony” (that is, no use of inbred animals coming from genetically controlled inbred colonies), the accumulation of natural mutation start. Since this moment, the number of point mutations in the genome of the colony increases and induces a genetic drift. Today, after several years of breeding, more and more teams face changes in phenotypes due to this phenomenon. This genetic drift appears each time a colony is bred heterozygously or homozygously. In addition, the impact of this drift is higher as most of the transgenic animal colonies start and live with a limited number of breeders pairs. It was demonstrated that with 10 pairs of breeders, the coefficient of inbreeding after 20 generation is 40%. To avoid this phenomenon, strategies could be applied to reduce or stop the genetic drift. These strategies are well documented in the literature of commercial suppliers including programs to maintain the genetic background of inbred colonies such as regular genetic refresh, permanent backcross, and genetic material freezing. Nevertheless, those strategies need to be used in the proper way to achieve the goal of managing the genetic drift of the models colonies and allow reducing results variability and evolution over time. This reduction of variability and evolution is a key of reduction of animal use in mice and rats models.

S36-1 Transgenic Mouse Colony Management

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Transgenic mouse facilities often experience common issues, including the lack of available cage space, inadequate post-approval monitoring (PAM), inefficient use of animals and outbreak of disease. These issues occur most often when multiple investigators are responsible for maintaining their own individual transgenic mouse colonies. The mouse facility at the Stowers Institute is maintained as a core facility that services the transgenic requirements of the Institute's principal investigators (PI). In addition to basic husbandry procedures, technical services such as breeding, weaning, tissue collection and injections are performed by a skilled team of animal technicians. Each PI is assigned an animal technician who becomes familiar with the lab's research goals and can help recommend and manage the breeding strategies. Together with the introduction of mouse data management software and outsourced genotyping, we have been able to double the number of studies performed with animals, without increasing the average daily cage census. Genetically modified mice can be produced inhouse at 15% cost, compared with commercial sources. Inhouse core colony animal production allows the investigators to order and use mice immediately, reducing the risk of introducing mouse pathogens and lengthy acclimation periods. Close communication between the animal technicians and a PAM specialist assures continued IACUC protocol compliance. Using this coordinated, centralized approach allows researchers to spend more time at the bench, limits unnecessary access to the animal facility and leads to more efficient use of animals.

S36-2 Archiving Genetic Altered Lines: When and How to Create Your Backup

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International repositories represent a fantastic tool to archive and to distribute genetically altered (GA) lines among the scientific community, but they are restricted to those lines that, after publication, represent a clear contribution to knowledge. A small percentage of the GA lines created every year reach this status. Daily functioning of any modern animal facility requires providing solutions to problems that can interrupt with devastating consequences the everyday work. Disease outbreaks; genetic contaminations, errors in breeding schemes or even natural disasters may compromise the work of several years. However, cryopreservation should not only be considered a safeguard for those situations, so far it is the only way to neutralize genetic drift. Cryopreservation can also represent a sensible way of managing lines when resources are scarce. To develop an in-door cryopreservation program is nowadays an achievable goal due to recent technical and scientific improvements that will be reviewed with special mention of the sperm freezing protocols developed at the Center for Animal Resources and Development (CARD), with high success rates in the previously frustrating C57Bl/6 background. Different techniques and less demanding equipment allow creating a back-up of the newly developed animal model still under study or represent an alternative to avoid losing valuable models when funding becomes a limiting factor that obliges to focus resources in fewer projects. Sperm, or embryos, that is the question to be discussed, but one way or the other the new strategies represent a small investment that, as car insurance, will prove invaluable when needed

S36-3 Genetic Contamination, There Is Always the Risk: The Importance of Genetic Monitoring

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Most of the genetically altered (GA) mouse strains present in our facilities are black! Their genetic background is either entirely or partially C57BL/6 derived. The sudden discovery of otherwise coloured offspring makes it easy to conclude that some degree of genetic contamination has occurred at some point during the breeding process. However, most

times the situation proves to be much more subtle, like when changes of the phenotype are gradually becoming apparent. So it is black alright, but are we still talking about the same sub-strain background? Are we sure that genetic drift is not playing up? Are we sure that the gene(s) of interest are still present? Are we sure other genes have not been introduced by accident? In summary: are we sure the model is still that model? How do you convince the owner of a strain where the gene of interest is in homozygous state, it is still important to genotype the nucleus breeders? An easy solution is to collect the tissue samples and to send them off to a commercial genotyping facility. Most of them will be able to provide you with the gene specific information and run a SNP panel for you. You may be fortunate and have such a facility at your institution, but most of us don't and do not have the resources and there is no easy way out. Are there practical solutions? Yes there are, albeit with some compromises.

WS08-1 Publishing Your Research in a Peer-Reviewed Journal

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Publishing in a peer-reviewed journal is a key part of the research process, enabling you to communicate your ideas and findings to the wider community and demonstrate impact within your field. Having a track record of published papers is also important for career development and therefore a crucial step for doctoral and early career researchers. For first-time authors the process can seem daunting, but there are lots of resources available to support and guide you: from choosing the right journal, preparing your manuscript for submission, navigating the peer review process, what to expect post-publication, ways to maximize the impact and dissemination of published work and information about publication ethics and your rights as an author.

WS08-2 Meet the Editor

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Laboratory Animals (LA) has seen some changes and it is therefore important to open the dialogue with affiliated societies, publisher and authors. The FELASA meeting provides the ideal platform for discussion, to compare point-of-views and sense the expectations of authors. The aim of LA is to publish a diverse range of papers, dealing with more optimal use of animals, management in biomedical research, or promote high quality education and training in laboratory animal science. LA particularly welcomes manuscripts reporting on Replacement, Reduction and Refinement, termed "3R" and focusing in protocols that either refine protocols involving animal models or improve experimental designs. Furthermore, the quality of publications is of importance to assure a high impact factor, to maintain the attractivity and guarantee citations of work published in LA. The Journal has well elaborated guidelines for authors and it is advised to adhere to them when preparing a manuscript. Mention ethical approval of (veterinary) authorities and indicate a respect of ethical guidelines. The ARRIVE guidelines are requested by many Journals to help improve reporting on animal research. It is advisable to use the Gold Standard Publication Checklist. Highlight the importance of findings, especially in the abstract. The experimental design and the statistical analysis should be detailed, particularly in relation using the appropriate numbers of animals. Check for grammar and typographical errors. Eventually, have the manuscript proofread if English is not your mother tongue. The Editors of LA make all efforts to decrease the turnaround time of manuscripts from submission to first decision.

WS08-3 What Is the Value of Systematic Reviews of Animal Studies?

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Within our research group SYRCLE (www.syrcle.nl), the current focus lies on systematic reviews of animal studies, because the execution of this methodology contributes to (i) higher quality science; (ii) more effective literature searches and thus implementation of the 3Rs; and (iii) higher patient safety. By performing systematic reviews it has been made transparent that a lot of details of animal studies are still not mentioned in scientific publications. In this respect, the journal Laboratory Animals has the best guidelines for publishing. Because of the lack of details in publications on animal studies, the Gold Standard Publication Checklist has been developed, intended to be used as a checklist already from the planning stages up until publishing of animal studies. For searching literature effectively, the step-by-step search guide, and two search filters have been developed. A more effective search of literature will prevent unnecessary duplication, and a thorough analysis of the literature will also lead to better implementation of the 3Rs. In order to stimulate the execution of systematic reviews and overcome obstacles, SYRCLE is dedicated to teaching, developing tools and guidelines and executing systematic reviews of animal studies. In my talk I will show several results of practical examples of systematic reviews of animal studies, illustrating the importance of analysis of already published work. http://www.syrcle.nl/

WS08-4 Ethics in Animal Research and Publishing

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Research with animals is in itself an ethically controversial issue and as such considered in guidelines and documents on publication ethics, albeit often in a rather minimalistic way. In scientific publication in general, major animal ethics issues include whether animal research as it is described in research reports conform to what is considered best practice, and major problems are insufficient consideration of refinement and insufficient details in reporting. The fact that most authors and readers of Laboratory Animals are experts in laboratory animal science and the 3Rs means that such issues do not frequently arise over manuscripts presented to the journal. However, a wide range of publication ethics issues can arise, ranging from 'salami slicing' (making as many publications as possible out of a single study) to controversy over the justification of a particular experiment. In this presentation, I will discuss ethical issues against the background of general advisory documents (for example, ICMJE http://www.icmje.org/urm_full.pdf) as well as my own and other colleagues' experience from the Editorial Board of Laboratory Animals. http://www.icmje.org/urm_full.pdf.

Poster Presentations

P001 Sentinels: Security for Laboratory Mouse Colonies

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In laboratory mouse colonies it is vital to preserve their health and the microbiological status because of the risk of infections, which may be inapparent and influence the results of experimentation or contaminate the biologic products derived from them. In 1983 Melvin Bosma published a scientific report that offers new perspectives to describe an immunocompromised mouse: CB -17. The aim of the study is to evaluate this model as a sentinel in a colony of laboratory mice. CB-17 mice wereused from a production bank, divided into numbers of 10 per breeding rooms at random, different lines were monitored together:

BALB/c, DBA/2, C57BL/6 and NMRI. It was examined viral, bacterial and parasitic entities for this species as recommended by the Federation of European Laboratory Animal Science Association. The most incident microorganisms in both sentinels and controls were *Pasteurella pneumotropica*, *Corynebacterium kutscheri*, *Entamoeba* spp., and *Syphacia* ssp. Out of the total microorganisms detected, 48.2% was found in greater numbers in sentinels than in controls, 34.4% was also detectable in both, and only 10.3% was more detectable in controls than in sentinels. The clinical signs observed in sentinel were due to respiratory syndromes compatible with the confirmation of the laboratory bacteriology and pathology. The use of the model was effective, recommended for its use in production colonies maintained in protected rooms, isolators systems and in genobanks, with an adequate security and identification, this would avoid the use of couples from the banks for health monitoring.

P002 Spontaneous Tumors Observed in Laboratory Primates during a 20-Year Period (1992 to 2012)

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A lot of information is available about tumors of commonly used laboratory animal species (rat, mouse, hamster). In contrast there are few reports on tumors of laboratory primate species. Up to the end of 2012 only about 40 neoplasms in cynomolgus monkeys (*Macaca fascicularis*) were published. Less than ten have been reported in common marmosets (*Callithrix jacchus*). Animals were fromtoxicity studies (control and dosed animals) conducted between 1992 and 2012. They were from breeders in China and Mauritius (cynomolgus monkeys), different European vendors (marmosets) or were from our own colonies. A complete necropsy was performed on all animals. Organ samples were fixed in 10% buffered formalin, embedded in paraffin wax, sectioned at a thickness of 5 mm and stained with H. E. Immunohistochemistry was applied for further classification of the tumors. Tumors were observed in 63 cynomolgus monkeys and 8 marmosets. The majority of cynomolgus monkey tumors observed was benign (49 benign compared with 14 malignant tumors) with 22 found in males and 41 in females. Most of the tumors (37) of the cynomolgus monkeys were seen in three organ systems (endocrine, respiratory and female genital). Marmosets revealed uterine adenocarcinomas, lymphosarcoma andC-cell adenoma. The histopathological and immunhistochemical features of the neoplasms will be described. With the present report we extend the current knowledge about the tumor spectrum of the two most common laboratory primate species by describing the tumor incidences and types observed in our facility during the past 20 years.

P003 Withdrawn

P004 Control of the Estrous Cycle in Guinea Pig (*Cavia porcellus*)

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The aim of this work was to look for a simple method to obtain synchronized ovulation in guinea pigs under farming conditions while respecting animal welfare. The luteolytic activity of three different prostaglandins F2α (PGF2α) analogs (D-cloprostenol, D,L-cloprostenol and Luprostiol) and a daily treatment with oral progestagen (Altrenogest) was tested successively at different stages of the estrous cycle on the same group of females during a period of 8 months. The estrous cycle length was not modified by the administration of prostaglandins, whatever the stage of the estrous cycle when the treatment was initiated. Our results led us to reject the use of PGF2α; analog to induce practical synchronization of the estrus in this species. In all the females (n = 29) given 15 days with Altrenogest (0.1 mL PO once a day), ovulation occurred 4.43 ± 0.13 days after the end of the treatment. Altrenogest treatment was followed by mating. No negative impact of the treatment on the pregnancy rates, delivery rates and litter sizes were observed. These results validate the first essential step of the development of a simple and reliable method of cryopreservation of strains of guinea pigs. This standard method of guinea pig estrus synchronization is less stressful towards the animals compared to techniques using progesterone tubing. The results of this work are currently used to secure lines of guinea pigs with high agronomic and economic value in Peru, and two unique laboratory lines used at the Institut Pasteur as animal models for the study of tuberculosis.

P005 Cesarean Rederivation of 12 Transgenic Mice Lines to Production and Experimentation Animal Service (SPEA) at Instituto de Biomedicina de Sevilla (IBiS)

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Embryo transfer has become the method of choice to obtain germ-free mice, relegating cesarean rederivation technique almost completely. However, depending on the infectious agents, this method can be an interesting alternative, because it allows reducing the number of animals needed in lines with low superovulatory response or limited effectives. At our old facility remained 12 transgenic mice lines positive for: *Helicobacter hepaticus*, *Helicobacter rodentium*, *Pasteurella pneumotropica*, *Trichomonas* spp., *Entamoeba* spp.). We report the rederivation to the new facility (SPEA/IBiS) by cesarean section. Fifty CD1 females were used as foster mothers. A total of thirty-eight pregnant transgenic females were sacrificed by cervical dislocation. Under sterile conditions uterine horns were excised and embryo sacs were recovered and immersed in 3% bleach solution at 37 ºC. In the barrier area, cardiac-breathing resuscitation techniques were performed. Once pups were revived and with healthy appearance, they were deposited with foster mothers. At weaning, offspring was marked and genotyped. Once colonies were established in their new location, they were health screened according to FELASA recommendations. Rederivation of eleven lines took four months, one line required two months more. Some of the genotypes originated in many cases early deaths in rederived offspring. The health profile results were negative for all microorganisms tested. SPF status was successfully achieved with 100% effectiveness. The SPF health category at the new facility was easily obtained and at low cost requiring a mean of 2-3donor females per line.

P006 Specific Detection and Differentiation of Rodent *Pasteurellaceae* by Multiplex PCR Using Primers Targeting the 16S-23S rRNA Internal Transcribed Spacer Regions

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Although FELASA recommends reporting of all rodent *Pasteurellaceae* present in the animal facilities, there are different opinions regarding the need to differentiate among the rodent *Pasteurellaceae* or at least among *Pasteurella pneumotropica* and the other *Pasteurellaceae*. We have previously demonstrated that the rodent *Pasteurellaceae* show species specific 16S-23SrRNA internal transcribed spacer (ITS) profiles. Here we report the development of a multiplex PCR assay able to detect on one hand all rodent *Pasteurellaceae* and on the other to differentiate *Pasteurella pneumotropica* biotype Jawetz, *Pasteurella pneumotropica* biotype Heyl and *Actinobacillus muris*, as the most prevalent members of the group. In this assay, a *Pasteurellaceae* common forward primer

based on a conserved region of the 16SrRNA was used in conjunction with reverse primers specific for all rodent *Pasteurellaceae*, *Pasteurella pneumotropica* biotype Jawetz, *Pasteurella pneumotropica* biotype Heyl and *Actinobacillus muris*, all targeting the 16S-23S ITS sequence. The specificity of the multiplex PCR was tested against 118 reference and clinical strains of *Pasteurellaceae*, including 34 strains of *Pasteurella pneumotropica* biotype Jawetz, 41 strains of *Pasteurella pneumotropica* biotype Heyl and 36 strains of *Actinobacillus muris*. In addition, other 8 mouse associated bacterial species which could pose a diagnostic problem were included. The assay showed 100% sensitivity and specificity. Identification of the clinical isolates was validated by ITS profiling and when necessary by 16SrRNA sequencing. This multiplex PCR represents the first molecular tool able to detect and differentiate among the *Pasteurellaceae* found in laboratory mouse in a single assay and may become potentially useful in diagnostic.

P007 Pinworm Detection in Individually Ventilated Cage Systems by Immunocompetent and Immunodeficient Soiled Bedding Sentinels

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Individually ventilated cage systems (IVCS) are routinely monitored with soiled bedding sentinels (SBS´s). Despite its advantages, the use of SBS´s may reduce the probability of detecting infestation compared with other monitoring methods. In particular, this fact is observed in pinworm infestation. Pinworm infestation remains common in laboratory rodent colonies. Two studies were conducted to evaluate the sensitivity of SBS´s immunodeficient NOD. CB17-*Prkdc*^{scid}/NCrHsd (NOD SCID) against two immunocompetent outbred strains, Hsd:ICR(CD-1) and RjOr1:Swiss(Swiss), in the detection of pinworms. Four different screening methods were used: perianal tape test, fecal flotation, plate method and histology. Both studies were performed in the same animal facility in a four month interval without a phamacological treatment between them. Positivity was considered if at least one of the techniques used was positive. One sentinel cage with five SBS´s (3 outbred + 2 NOD SCID) was used for each of the seven ventilated racks in both studies. In the first study only 17% (n = 18) of CD-1 compared with100% (n = 11) of NOD SCID could detect the presence of pinworms. In the second one, 81% (n = 21) of Swiss were positive compared with 100% of NOD SCID (n = 14). Comparing the detection rates of the four techniques, they were higher in NOD SCID sentinels. NOD SCID sentinels showed an extremely high efficiency in pinworm detection. As the objective of a health surveillance program is the early and reliable detection of the infectious agents who affect research, immunodeficient sentinels should be considered in routine detection, eradication and quarantine programs in order to detect pinworm infestation

P008 Improvement of Routine Health Monitoring in IVC Systems by Analysis of Exhaust Air Dust Samples

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Health monitoring of rodents in IVC systems is a challenging task. Each single cage represents a separate hygienic unit, if cage and mice are handled properly. Routinely, used-bedding sentinels (UBS) are examined every three months, but limitations of this method are obvious: impossibility of aerogen transmission, uncertain transmission of unwanted organisms by used bedding and unclear susceptibility of UBS. In order to improve health monitoring in IVC systems, we have developed a reliable and easily practicable method of testing excreted unwanted organisms in the exhaust air prefilter of an IVC rack. Murine norovirus (MNV), which still has a high prevalence in many experimental animal facilities, was used as a representative unwanted organism in our tests. In an experimental colony with a defined prevalence of MNV, exhaust air prefilters were tested weekly for MNV with RT-qPCR of dust samples and results were compared with UBS serology. We could demonstrate that at low MNV-prevalence, UBS passage MNV nucleic acids without being infected and thus seroconversion of UBS could not be detected. By analyzing exhaust air pre-filters, however, MNV could be reliably detected after just one week with a minimum of only 5 MNV-positive mice per IVC rack. Several 12-week-testing periods (5 at the time of abstract submission) showed clear superiority of exhaust air prefilter PCR in comparison to UBS serology. For routine use, we have designed a device which can easily be attached to the exhaust air prefilter and allows an uncomplicated collection of exhaust air samples.

P009 Cases of Distal Duodenal Ulcers in Common Marmosets

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Recently, biomedical research using common marmosets (*Callithrix jacchus*) has increased, including studies of neurologic diseases and regenerative medicine. However, there is insufficient information on diseases of common marmosets. Here, we report 27 cases of deep ulcers at the duodenojejunal flexure seen in common marmosets over the past 4 years. The clinical symptoms included continuous vomiting and subsequent weakening. Necropsy findings showed luminal dilation of the stomach and duodenum, accompanied by intestinal stenosis at the duodenojejunal flexure. The histopathological changes were similar in all cases. Stomach tissues, including the pyloric region, were normal. The histopathology of the duodenum included gastric epithelium metaplasia and loss of tissue that reached the muscular layer and cicatricial strictures in the duodenojejunal flexure. We have not yet detected any pathogenic organisms in these cases. To our knowledge, this is the first report of distal duodenal ulcers in nonhuman primates. Peptic ulcer disease usually arises in the distal stomach or proximal duodenum. Even in humans, distal duodenal ulcers are rare. The duodenum of common marmosets curves sharply at the duodenojejunal flexure. We postulate that unknown factors caused acidification in the duodenum, disrupting the membrane defenses. We also believe that physical factors at the duodenojejunal flexure are involved in the development of distal duodenal ulcers.

P010 Rabbit Colony Infected with a Bovine-Like G6P[11] Rotavirus Strain

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Group A rotaviruses (RVA) are the main etiological agent of infantile diarrhea both in humans and animals worldwide. A limited number of studies have investigated the molecular characteristics of RVA strains in stool specimens of rabbits, with only a few lapine RVA strains isolated and (partially) characterized to date. The most common G/P-genotype combinations found in rabbits are G3P[14] and G3P[22]. In this study a RVA strain was isolated from the small intestine of a 9 weeks old rabbit from an infected laboratory rabbit colony. The RVA strain RVA/Rabbit-tc/NLD/K1130027/2011/G6P[11] was shown to possess the typical bovine G6P[11] genotypes. The complete genome of this unusual lapine strain was sequenced and characterized. Phylogenetic analyses of all 11 gene segments revealed the following genotype constellation: G6-P[11]-I2-R2-C2-M2-A13-N2-T6-E2-H3. The VP1, VP2, VP3, VP6, NSP2 and NSP4 genes all belonged to DS-1-like genotype 2, but clustered more closely to bovine RVA strains than to lapine RVA strains. The NSP1 genotype A13 is typically associated with bovine RVAs, while the NSP3 genotype T6 and the NSP5 genotype H3 have been found in a wide variety of species. However, the isolated strain clustered within bovine (-like) T6 and H3 subclusters. Overall, the data indicate that the RVA strain is most closely related to bovine-like RVA strains and

most likely represents a direct interspecies transmission from a cow to a rabbit. Altogether, these findings indicate that a RVA strain with an entirely bovine genome constellation was able to infect, spread and cause disease in a laboratory rabbit colony.

P011 Infection of a Mouse Colony with Mouse Parvovirus through Food

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An infection with Mouse Parvovirus (MPV) was found in an animal facility by routine health monitoring. Wide scale screening of the total colonies of this facility revealed that the infection was predominantly found in mice which received a certain not-radiated diet food suggesting the root cause of this infection. Detection of MPV by PCR demonstrated the presence of the virus in the food. An experimental study to proof the direct relation of the presence of MPV in the food and infection of the laboratory population is currently being set up. This is the first demonstration of detection of mouse parvovirus in food.

P012 Application of Molecular Technologies on Noninvasive Samples to Enhance Health Monitoring Programs of Rodents in IVCs, a Comparison with a Traditional Approach

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Individually ventilated cage (IVC) systems are widely used to protect the health status of laboratory mice. Meaningful sampling of the IVC-housed population to establish the health status is challenging as each cage may be its own microbiological entity and so sentinel programs are often deployed. Sentinel programs are prone to yielding false negative laboratory results due to compromises in sampling. This study compares immunocompetent outbred mouse sentinels (CRL:CD1) exposed to soiled bedding using laboratory methodologies typically described in FELASA health monitoring recommendations, against non-terminal sampling from pairs of immunodeficient and immunocompetent outbred mouse sentinels (Crl:CD1-*Foxn1nu/nu* and Crl:CD1-*Foxn1 nu/+*), using an array of real time PCR tests and a serology panel. The study was designed around ten single-sided IVC racks of a defined health status managed as a unit in one facility spread over three rooms and involved two sample points each being submitted to two independent laboratories. The exposure of immunodeficient mice to soiled bedding with nonterminal sampling of those mice enabled the detection of *Helicobacter* species, pinworms and various opportunistic bacteria. In contrast, the traditional exposure and detection methods were consistently negative at each time point. No clinical signs were recorded in any of the immunodeficient mice. These data indicate the use of a more targeted soiled bedding program exposing immunodeficient mice combined with molecular diagnostic approaches can be more sensitive for prevalent agents. Molecular diagnostics also support refinement through the shipping of clinical nonterminal samples rather than animals.

P013 Is It Possible to Eradicate the Mouse Parvovirus from a Laboratory Mice Colony, Using Only Strict Procedures of Colony and Sanitary Management?

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In March 2010, one of our commercial supplier informed us that our facility had received mice with active parvovirus infections from one of their production rooms. The presence of this virus in an animal facility is problematic because the parvovirus induces persistent infection in mice and it is also difficult to eradicate since it has an environmental stability. For all these reasons, it is usually suggested the euthanasia of all animals, both infected and potentially infected, in order to found new animal colonies. In our animal facility we did not use this strategy though, because most of the parvovirus positive mice were used in important experimental protocols. However, an eradication of the virus was needed. Therefore, we decided to actualize an alternative plan of action, working exclusively on strict procedures of colony management: for example we decided the selective euthanasia only of a few infected mice and the breeding cessation, we moved the infected animals in a provisional unit outside the animal facility and we had severe procedures of cleaning and disinfection of the environment carried out. The health condition of the mice has been frequently monitored in order to constantly control the trend of the parvovirus diffusion. After 9 months, the parvovirus was no longer present in our health report and even now the parvovirus is absent. Therefore, our results indicate that there is a possibility to eradicate a viral infection only using strict procedures of colony and sanitary management, without the elimination of the whole animal colony

P014 Molecular Diagnostic Tests for Detection of Microbial Pathogens in Laboratory Zebrafish

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Zebrafish are being used increasingly as a model organism in biomedical research. With the expansion of zebrafish into new areas of biomedical research, there is an increased risk of confounded or invalid experimental results due to underlying infections. Improvements in the health status and health monitoring of zebrafish colonies are therefore important objectives in zebrafish facility management. In contrast to rodent facilities, relatively few zebrafish facilities have health monitoring programs in place to detect the naturally occurring pathogens of zebrafish. To date, health monitoring of zebrafish has relied heavily on traditional methods, including histopathology, impression smears, squash preparations, and wet mounts. In this presentation, we report development of a panel of molecular diagnostic assays that can be used for health monitoring of zebrafish colonies. Developed molecular assays include assays for five parasites, *Pseudoloma neurophilia, Pseudocapillaria tomentosa, Piscinoodinium pillulare, Pleistophora hyphessobryconis,* and *Ichthyophthirius multifiliis,* as well as four bacterial pathogens, *Edwardsiella ictaluri, Mycobacterium chelonae, Mycobacterium haemophilum*, and *Mycobacterium marinum* and the virus, Infectious spleen and kidney necrosis virus (ISKNV) For each molecular diagnostic assay, the lower limit of detection was determined to be 20 or fewer template copies. Clinical validations studies comparing the results of molecular assays with traditional diagnostic methods were also performed. Results demonstrated that molecular diagnostic assays were more sensitive than traditional methods. The availability of these molecular assays for health monitoring will allow improved health status and health monitoring of zebrafish colonies.

P015 Boone Cardiovirus, a New Cardiovirus Found in Laboratory Rats

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This study reports a newly identified virus found in the feces of laboratory rats. Complete sequence of the 8.5 kb genome indicated that this virus represents a new species of *Cardiovirus* that until now has not been detected. The virus has been provisionally designated as Boone cardiovirus (BCV). Phylogenetic analysis indicated that BCV is closely related to Rat theilovirus (RTV) and Theiler's murine encephalitis (TMEV). Comparison of the protein sequence to RTV and TMEV

revealed that BCV shares <45% amino acid identity in the polyprotein region and <50% amino acid identity in the P1 capsid protein containing region. To assess the prevalence of BCV in laboratory rats, a Multiplex Fluorescent Immunoassay (MFI) specific for BCV was developed. Evaluation of rat sera from diverse geographical regions revealed a prevalence of 2%, very similar to that found for RTV. There are no apparent correlations of BCV infection with age, gender or rat strain. When tissues from rats were evaluated for BCV by RT-PCR, BCV was predominately detected throughout the GI tract and was persistently shed in the feces. Runting, hemorrhage and neonatal deaths have been observed in some BCV infected rats, but to date studies to confirm cause and effect have not been performed due to the inability to grow the virus in vitro. In conclusion, these studies demonstrate the existence of BCV, a previously undetected cardiovirus, which is persistently shed in the feces of laboratory rats. Additional studies are underway to determine the impact of infection on research.

P016 Pathogenicity of 3 *C. bovis* Strains in Nude Mice

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Corynebacterium bovis causes hyperkeratosis and acanthosis in immunodeficient mice. We compared three genetically distinct strains of *C. bovis*: a field isolate (HAC) from symptomatic nude mice, another field isolate from asymptomatic mice, and one of bovine origin (ATCC 7715). Nude mice were inoculated with pure cultures of one of the 3 strains; each strain was used in a separate isolator. Bacterial population dynamics, as well as gross and microscopic lesion production, was assessed for 5 to 12 weeks using a 16S rRNA-based real-time PCR assay, bacterial culture and histopathology. We found no significant differences in pathogenicity among the strains. Additionally, no correlation was found between the hair follicle growth stage, assessed microscopically, and *C. bovis* population levels or lesion production, nor did housing in filter-top cages (higher humidity) compared with open-top caging alter lesion development. The study reiterates the low morbidity often associated with *C. bovis* infection, as well as the higher frequency of mild histologic lesions relative to gross lesions (clinical signs). Bacteriology was sensitive for detecting *Corynebacterium* spp., although automated identification systems occasionally incorrectly speciated *C. bovis* and few related species. PCR of skin swabs was not only sensitive, but more specific than culture for detecting *C. bovis*. Importantly, feces were found to be equivalent to skin swabs as a PCR sample for detection of *C. bovis*. Screening of customer-submitted samples from vivaria across North America using PCR for the rpoB gene to distinguish among strains revealed that the HAC strain is the predominant *C. bovis* strain detected in nude mice.

P017 Enteropathogenic *Escherichia coli* Infection with Gastrointestinal Hemorrhage in the Common Marmoset

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Enteropathogenic *Escherichia coli* (EPEC) is commonly isolated from bloodystool samples from common marmosets. In this study, we examined theepidemiological relationship between gastrointestinal hemorrhage and EPEC infection in this species. A total of 609 stool samples (bloody, 99; other samples, 510) were cultured using DHL agar and XM-EHEC agar as a primary test. PCR for eae gene was performed on the isolates. Pulsed-field gel electrophoresis (PFGE) was performed on 50 selected EPEC isolates (bloody stool samples, 30; other samples, 20) in order to determine anygenetic relationship among the isolates. EPEC was isolated from a significantly higher proportion of bloody stool samples than from other samples (52 from 99, 52.5% compared with 80 from 510, 15.7%). The 50 selected EPEC isolates showed a similar pattern on PFGE. These results indicate that EPEC is associated with gastrointestinal hemorrhage in the common marmoset. Further investigation is necessary to determine the pathogenicity of EPEC in this species.

P018 Prevalence of Rodent Viruses and *Mycoplasma* in Cell Lines and Other Biologically Based Reagents

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Cell lines and biologically derived reagents such as serum, conditioned media and antibody solutions can potentially be contaminated with rodent pathogens. Once introduced to study animals, these contaminations can spread to other animals and interfere with research. We retrospectively compiled results of molecular diagnostic screening of cell lines and other biologic reagents over eight years of testing in our laboratories. All screening employed real-time PCR capable of detecting less than 10 copies of each targeted gene in a sample. Controls were included to assess adequate recovery of nucleic acid and test for the absence of PCR-inhibitory substances. Approximately 4,000 to 4,500 samples were tested for an extensive panel of viruses (rodent and human). The submitted sample types included antibody solutions, various cell lines, tumor lines, culture matrix solutions and various human carcinomas. The results showed positive samples for lymphocytic choriomeningitis virus (LCMV), lactate dehydrogenase elevating virus (LDV), parvovirus, polyomavirus, reovirus, mouse hepatitis virus (MHV) and *Mycoplasma* spp. As for human viruses, the results showed positive samples for hepatitis B, human papillomavirus type 18 (HPV18) and epstein barr virus (EBV). Overall, more than 1% of all samples were found positive for rodent viruses. Less than 0.1% of the human-labeled samples were found positive for rodent viruses. More than 7% of all samples (rodent and human combined) were found positive for *Mycoplasma*. As evidenced here, biologics are a very real threat to the biosecurity of a laboratory animal facility. PCR is currently the primary method for biologic screening.

P019 Assessment of Whole Body Oxygenation under Different Anesthetic Conditions by near Infrared Spectroscopy (NIRS) Technology

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Near Infrared Spectroscopy (NIRS), is a technique for the evaluation of surface organ oxygenation. Briefly, NIR light (650 to 1050 nm) penetrates into tissue and it is partially absorbed by tissue chromophores such as hemoglobin (Hb) + myoglobin (Mb) in their oxy- and deoxy-forms (920 and 760 nm, respectively) and partially scattered. The difference in absorptions is used by a NIR-sensitive camera system to calculate the amount of oxy-(Hb+Mb) and deoxy-(Hb+Mb) and then ratio of oxy-(Hb+Mb) to total-(Hb+Mb). Indeed, the captured intensity map normalized to a reference map provides a pseudo-absorbance spectrum, which can yield the relative contents of the chromophores and an oxygen saturation parameter (OS). Processing software generate 2D OS map automatically, in real time (acquisition time < 1 s). The main limitation of this method is mostly associated with the finite depth of light penetration into tissue, which allows examination of only superficial layer (ca.4 mm thick). This study aims to evaluate whole body and regional perfusion and oxygenation, by a NIR-sensitive camera (Kent imaging Inc. Canada), during different general anesthesia protocols. Both intraperitoneal and gaseous general anesthesia, with or without mechanical ventilation and different values of FiO2, will be tested

during different invasive surgery procedures as thoracotomy and laparotomy. The NIRS parameters are then correlated with breath and heart rate, body temperature, systemic blood pressure and blood clinical biochemistry for complete characterization of each anesthetic protocol.

P020 A Case of *Trueperella pyogenes* Isolated from Several Infected Skin Wounds in Gottingen Minipigs

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Trueperella pyogenes is a common inhabitant of the mucus membranes of healthy animals and is one of the most opportunistic pathogen of domestic pigs causing a variety of suppurative infections involving the skin, joints and organs. The microorganism expresses virulence factors that contribute to its pathogenic potential although the role of the bacterium in the infections is still unknown. In this report we describe clinical cases of skin abscesses in conventional housed female minipigs produced by *T. pyogenes*. The abscesses appeared after skin injury due to surgery, intramuscular injection or fights. Few cases did not require intervention. The animals that needed treatment were anesthetized and the infected zones were cleaned with chlorhexidine. A short sterile incision with scalpel was performed allowing a proper pus drainage with diluted povidone-iodine rinsing. The wounds were covered with a sterile pad and dressing tape. In case of relapse, the animals underwent surgery in order to debride the abscess capsule and the surrounding necrotic tissue in an open approach. Postoperatively, meloxicam was administered every 24 hours as needed. Samples of the pus were taken in using a hyssop in order to isolate the pathogen and run an antibiogram. Abundant and pure colonies of *Trueperella pyogenes* were identified in all cases. Antibiotherapy with long action amoxicillin was initiated. The wounds healed completely in a pair of weeks. The infected wounds did not recover without treatment and, in serious cases, an open surgery to remove the pus and debridate the necrotic tissue had to be performed.

P021 Hematological and Biochemical Reference Parameters in Cynomolgus Monkeys (*Macaca fascicularis*) from Philippines

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Cynomolgus monkey is a nonhuman primate species widely used in biomedical research, such as for xenotransplantation studies. Knowledge of its hematologic and biochemical reference parameters and their variability due to age is essential to improve clinical decision-making and postsurgical treatment management. The goal of this preliminary study was to determine reference ranges in relation to age for hematological and biochemical parameters in 97 healthy, socially housed, cynomolgus monkeys. Purpose-bred animals, both males and females, were aged 21 to 112 months, weighted 1.7 to 5 kg, and originated from the same breeding background in Philippines. Blood samples were collected between 2007 and 2011 during routine health checks. All samples were analyzed in a standardized diagnostic laboratory equipped with 5diff hematology analyzer and a new generation clinical chemistry analyzer. Analyses were performed in 0.2 mL K3-EDTA and in 0.3 mL Li-heparin blood specimens. For the purposes of age group comparisons, subjects were divided into four age classes: 21 to 36 months (25.8%), 37 to 42 months (26.8%), 43 to 54 months (24.7%) and 55 to 112 months (24.7%). Several parameters demonstrated to be significantly influenced ($\alpha = 0.05$) by age: MCH, platelets, MPV for hematological parameters and albumin, creatinine, alkaline phosphatase (ALP), globulin, total protein, glucose, phosphate and urea for biochemical parameters. Platelet values were the highest in the oldest group, whereas in the same group ALP, phosphate and urea were significantly lower than in the other three groups. In conclusion, blood parameters age specific intervals may be useful to manage the clinical and postsurgical care of animals involved in transplantation studies.

P022 Pancreatic Enzyme Levels Changes after Double-Balloon Enteroscopy

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Double-balloon enteroscopy (DBE) is an endoscopic technique for the diagnosis and treatment of small intestine diseases, that is considered a safe and well tolerated, but an increase of pancreatic enzymes and pancreatitis are recognized as potential complications of the procedure. This study aimed to assess the effects of the EDB technique on the pancreas at different times by the analytical determination of the following parameters: pancreatic lipase, amylase and C-reactive protein. The study was carried out in 25 Large white pigs divided into three groups: 5 control animals, 10 animals that underwent EDB during 90 minutes and the remaining 10 who underwent EDB during 140 minutes. Blood sampling and analytical tests were performed at the following times: baseline, end of the scan, 24 h after the procedure and at 7 days post procedure. All groups showed a very significant increase in all three parameters 24 h after the technique, finding lower values close to the baseline 7 days after the procedure. No differences were found between the study groups at the different times. The double balloon enteroscopy (EDB) produces a significant increase of pancreatic enzymes that is reversible hours after the procedure; this increase does not depend on the duration of the exploration according to the parameter studied in our work.

P023 The Case of a Familial Form of Autoimmune Myasthenia Gravis in a Skinny Pig From a Private Breeding

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The Skinny Pig (*Cavia aperea f. porcelus*) is an almost hairless breed of guinea pig. Myasthenia gravis is an autoimmune disorder of neuromuscular transmission involving the production of autoantibodies directed against the nicotinic acetylcholine receptors (AChR) and leading to muscle weakness and fatigability. The adult 3-year-old female Skinny Pig died after showing symptoms of the lameness of the pelvic limb, which progressed to paraparesis and tetraparesis, followed by anorexia, dyspnoe and general weakness. Two years later, the same breeder arrived at the clinic with two more Skinny Pigs female (3 and 4 years old) with very similar symptoms. One animal died during the examination, and the other twenty hours later. All three females were related. The blood from the third guinea pig was collected and tested for the presence of antibodies against the AChRs. In all three cases, the autopsy was performed and samples of the thymus, esophagus and hind limbs muscles were fixed in 10% neutral buffered formalin, embedded in paraffin and stained with hematoxylin and eosin (HE), iron hematoxylin - picric acid - acid fuchsin (van Gieson) and hematoxylin - basic fuchsin - picric acid (HBFP). The postmortem examination revealed the pale pink limbs muscles with a significantly decreased fiber diameter, sagging walls of the esophagus and a tumor of the thymus. Histologic studies showed atrophy of limbs muscle fibers with mild inflammatory infiltration and necrotic focus, distinct atrophy of the muscular membrane, fibrosis and mild inflammatory infiltration in the esophagus, and thymoma.

P024 Citrolive: A New Natural Functional Ingredient Helps against Obesity, Cardiovascular and Nonalcoholic Fatty Liver Disease

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Obesity is a worldwide metabolic disease associated with the onset of several pathologic conditions such as type 2 diabetes, dyslipidemia, coronary heart disease, cancer, respiratory complications, nonalcoholic fatty liver disease (NAFLD), osteoarthritis, and also neurodegenerative diseases. In different studies polyphenols have shown a body weight loss and antihypertensive effect. In this study we show results of a specific combination of citrus fruits and olive leaves extracts, named Citrolive, as a potential support in treatment of obesity.30 female of BKS-db/db ($Lepr^{db}$ /$Lepr^{db)}$ mice were used. Data were collected during 7 months. Animals were randomly distributed in 3 groups:
* Control * SC-Cit: Citrolive subcutaneously injected (1mg/day)
* PO-Cit: Citrolive orally dosed (5mg/day)At the end of this study mean body weights were: 46.5 g, 51.5 g, and 39.7 g for the control, PO-Cit and SC-Cit groups respectively. A higher decrease of body weight in SC-Cit was confirmed either through a higher level of adiponectin or a lower level of TNF-α compared with control and PO-Cit groups. Hypertension was also significantly lowered in SC-Citrolive group. Liver steatosis was assessed microscopically showing a 48% (level 2) for control group, a 36% (level 2) for PO-Cit and a 17% (level 1) for SC-Cit. PO-Cit group showed higher macrovesicular steatosis. SC-Citrolive treatment showed a beneficial effect on weight loss, control of hypertension and NAFLD level. Further pharmacokinetic studies will be necessary to establish the differences between the two routes of administration of Citrolive.

P025 Detection of the Segmented Filamentous Bacteria by PCR

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Segmented Filamentous Bacteria or SFB, is a host-specific, commensal bacterium provisionally named *Candidatus savagella.* SFB inhabits the intestinal epithelium of wide range of host species, including mice and rats. The bacteria form long filaments in the small intestinal lamina propria of the rodent gut that often span the length of several villi. To date, efforts to culture SFB in vitro have been unsuccessful, which has hindered studies aimed at understanding the biology of this organism and its pathogenesis. However, recent studies have shown that SFB colonization of mice can have profound effects on the immune system, including induction of IL-17, enhanced resistance to intestinal bacteria and increased autoimmune responses. These findings have prompted a renewed interest in SFB and detection of SFB. Historically, detection of SFB required euthanasia of the animal and histologic evaluation of the small intestine for the presence of the characteristic long, filamentous bacteria attached to the epithelium. In this study, we report the development of an antemortem PCR assay to detect SFB using fecal pellets with has a sensitivity of 20 template copies. Side-by-side comparison documented improved sensitivity of the PCR assay over traditional histologic evaluation methods. The newly developed fecal PCR assay for SFB provides, for the first time, an antemortem method for detection of SFB, which will be useful in documenting the presence or absence of this organism in future studies.

P026 Reversible Leukoencephalomyelopathy in Specific Pathogen-Free (SPF) Cats, Fed with γ-Irradiated Cat Diet

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This report describes a spontaneous outbreak of leukoencephalomyelopathy in a European commercial SPF laboratory cat breeder, and suggests a possible association with long-term feeding with a γ-irradiated commercial diet. The outbreak affected a 20% of the colony, progressing through 3 months. Both sexes were affected without sharing ancestries. Initial presenting clinical signs included swaying, difficulty in landing or jumping and proprioceptive defects. Itinerant hind limb paraparesis and ataxia worsened in 4 cats (3%) that developed a severe paraplegia. Initial neurologic examination suggested an involvement of spinal cord disease. Different diagnostic approaches were undertaken but not abnormalities were found in hematology, biochemistry, serology of infectious diseases (FCV, Chlamydia, FeCV, FIPV, FVR, FHV-1, FIV, FTLV, FeLV, FPLV and toxoplasmosis), heavy metal toxicosis, CSF analysis and spinal radiography. Computer tomography and muscle/nerve biopsies focuses the problem. Necropsy of two severely affected animals, histopathology and electronic microscopy confirmed an acquired demyelination process affecting the Central Nervous System while excluding infectious and other possible diseases. Results suggested a toxic/nutritional ethiology. Treatment was established with vitamins supplementation (B1-B6-B12, A, C and E), taurine and potassium while a pasteurized tinned diet was used. No further cases occurred and the rest of affected animals except one improved and normalized in 4 months. We concluded that γ-irradiated dry diet used to feed the SPF cats leads to free-radical formation that may play a determinant role in the presentation of the disease. High-level γ-irradiated food should not be used as cat diet due to the risk of severe neurologic dysfunction.

P027 Antemortem Detection of Fur Mites by PCR

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Traditional methods for diagnosis fur mite infestation rely on antemortem or postmortem microscopic examinations of hair or pelt to visualize mites or mite eggs. These methods are labor intensive and may lack the sensitivity needed to detect low level infestation. In this study, we developed a fur mite PCR assay, evaluated its sensitivity and specificity, and compared its performance to traditional diagnostic techniques. The PCR assay targeted the rRNA gene, had an analytical sensitivity of < 10 copies of fur mite DNA, discriminated between *Mycoptes musculinus* and *Myobia musculi* or *Radfordia* spp., and did not detect grain or environmental mites. To evaluate the utility of PCR for the detection of fur mite infestation, fur mite-free outbred mice were housed for 4 days with mice infested with *Myocoptes musculinus* and *Myobia musculi* and tested for fur mites using several antemortem diagnostic tests. PCR of skin swabs was positive in 14/15 mice; whereas, only 10/15 mice were positive by fur pluck examinations and only 5/15 mice were positive by tape tests. Postmortem pelt examinations revealed fur mites in 12/15 mice. In addition, a single swab was used to sequentially sample the bedding or inside perimeter of a soiled cage that housed mite-infested mice and nine soiled cages that housed mite-free mice. All composite swabs tested positive for fur mites by PCR regardless whether the infested cage was sampled first ($n = 10$ replicates) or last ($n = 7$ replicates). These results show that PCR was the most sensitive diagnostic test for detection of fur mite infestation.

P028 Direct Sampling of Quarantined Mice Accurately Reflects Their Health Status and Shortens Quarantine Time

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Quarantine programs for imported mice may last up to 12 weeks

when the method of soiled bedding transfer to sentinel mice is used to screen for pathogens. Decreased quarantine time may result in failure to detect pathogens and placement of diseased mice into the vivarium. A 4-week study was performed to determine if results of testing samples directly from quarantined mice would accurately reflect their health status. Accurate results would allow decreased quarantine time. Female 4 to 5 week-old outbred mice naturally infected with MHV, TMEV, MNV, MPV, *Helicobacter*, *Pasteurella pneumotropica,* furmites and pinworms were divided among 10 cages, 2 per cage. During the second week of quarantine, feces and fur swabs were collected from quarantined mice for PCR testing and oral swabs for culture. Sentinels were 4 to 5 week-old CD-1 female mice housed in 5 cages, 2 per cage. Each sentinel cage received soiled bedding from 2 quarantine cages weekly for 3 weeks. At study end, samples from sentinel and quarantined mice were collected for viral serology, *Helicobacter* PCR, traditional parasitology tests and oral swab culture. Results from samples collected from quarantined mice at week 2 of quarantine correlated 100% to the infection status determined at the end of the study. Results from samples collected from sentinels revealed a lack of transfer of MNV, *Helicobacter*, fur mites and *Pasteurella pneumotropica* and ineffective transfer of pinworms and TMEV. In conclusion, testing samples directly from quarantined mice provide an accurate reflection of their health and can be used to dramatically shorten quarantine.

P029 Health Monitoring of a Nonrodent Colony in India

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Over period of time, many nonrodent facilities in India encountered deadly viral infection (Canine parvovirus) and zoonotic (Leptospirosis) diseases. These incidents forced them to seize their preclinical studies and facilities. As our company started its project in India in 2011, which is the only nonrodent (Beagle dog) commercial breeding facility in India, we are obtaining the required samples from our animals to meet the requirements of pre-clinical research industry of Europe and all over India. Breeding correctly with highest animal welfare and quality standards are not the only factors which Indian authorities and Pre-clinical research industry demands, but also the highest quality monitoring of animals. The highest quality animals are required in the research experiments to avoid infections interfering with the outcome of the experiments, and this quality is always related with the quality of the care and husbandry practices. Hence, a system was required to monitor the health status of the nonrodents to evaluate and characterize the microbiological status of the colony as per Indian epidemiology by examining animal samples for the presence of the infectious agents. It is concluded that if the proper preventive measures and health monitoring systems like as per FELASA recommendations are incorporated into the system of husbandry and care, it is possible to sustain high standard conventional breeding colony of nonrodents in India. The health monitoring system based on FELASA recommendations was very effective also, it will gather many useful information about the animal and disease controlling strategies in an Indian nonrodent facility.

P030 The Effects of Foster Mother Care on Behavior Parameters in Rat Pups

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Maternal care and nutritional deprivation of rat pups, especially the first 10 days of their lives, causes nonhealthy development or loss of the pups. Considering this situation, the most ideal method which will ensure the healthy development of the pups should be investigated. Therefore, by carrying out this study on conventionally bred rats of our facility, we compared the behavior parameters of foster-mother bred and biologic mother bred pups. For this study, 8 adult Wistar albino pregnant rats were grouped into 3 as fallows; in the first group there were foster mother raised pups; in the second group there were pups which are nurtured by their biologic mother; in the third group there were pups which were nurtured by their biologic mothers but were carried to another cage as if their mother was changed. These pups were tested in elevated plus maze, open field test and Morris water maze respectively starting from age of 8 weeks. The statistical differences of pups between groups and sex were evaluated using ANNOVA. This study has ethical approval from Dokuz Eylul University Local Experimental Animal Ethical Committee. In literature no similar study was found. By the results that are obtained from this study, the care and housing protocols of breeding unit of animal facilities will be modified to increase the welfare of the pups raised by foster mother.

P031 The Common Endoparasites of Laboratory Mice

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The health monitoring of animal facilities is now widely generalized. In our institution, we receive laboratory rodents from a large number of facilities in France to perform health controls. We can observe a large scale of health status namely concerning endoparasites. We found here it was a good idea to show to the customers what do a pinworm or a *trichomonas* look like. In the first part, we will explain how to prepare the slides to be able to identify correctly the different species. In a second part, we will present a photo gallery of what we usually find during the health controls of the laboratory mice we receive at our institutions. We will give you some tricks to be able to recognize the different species.

P032 NSG Mouse Strain, Health Monitoring, and Microbiological Findings

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The NSG strain is one of the most immunodeficient mice available, and provides an effective model for studies where the engraftment of human cells is required. But because of their severe adaptive and innate immune deficiency, they must be kept under high environmental control standards. Routine health monitoring, according to FELASA guidelines, included antigen testing in NSG animals and antibody testing in sentinel animals. We found saprophytic flora, when performing classic microbiology techniques, in different tissues and organs. Many of the sampled animals had positive growth in the spinal cord, stifle joint, heart, spleen, liver, kidney or blood. In order to discard possible tissue contaminations, we sent samples to three different external laboratories. All the samples submitted came back with the same results. We postulate that the spreading of these saprophytic bacteria may be due to a lack of IgA secretion at the mucosa epithelium, and their dwelling in those sites to the immunodeficiency including the impaired macrophage activity. The potential clinical significance of these isolates in NSG mice, if any, is not known. To explore the possible connection between these bacteria and animals with signs of slight limb numbness and paralysis and arthritis, seen in 0.5-1% of them, further studies are needed.

P033 Storage Disease with a Glucidic Component in PVG C6-/- Rats: A Clinical Case Report

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In the colony of mutant PVG C6 -/- rats (complement factor 6 deficient) bred at our conventional facility, pathologic pictures due to systemic storage disease were found. The patients, predominantly females, showed signs of abdominal distension. Furthermore it was observed neonatal mortality near 100%. At necropsy it was observed hepatosplenomegaly, small intestine wall thickening (similar to "rubber tube"), enlargement of abdominal and thoracic lymph nodes and point or irregularly shaped lesions in the lungs. In all interested organs, the histologic analysis showed large amounts of voluminous cells, similar to macrophages, characterized by cytoplasmic heavy accumulation of an undefined substance. On these findings the hypotheses were formulated of mycobacteriosis or lysosomal storage disease. The presence of mycobacteria was excluded after specific histochemistry analysis (Ziehl-Neelsen staining). It was highlighted instead a generic positivity for carbohydrates (PAS staining) in all interested organs, while detection of glycogen (PAS-diastase staining) and mucopolysaccaride (Alcian pH 2.5 staining) proved negative. In conclusion specific stainings have shown that the accumulation of cellular material has a carbohydrate component, suggesting a lysosomal storage pathology generally caused by enzyme deficiencies. Additional investigations (electron microscopy, biochemical tests on fresh tissue) would be required to ascertain the exact substance composition.

P034 Assessment of Body Temperature in Rats Using Infrared and Rectal Temperature Probe

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Infrared thermometry consists of a simple, rapid, noninvasive method of body temperature measurement that reduces the stress of the animal. The purpose of the certain study was the evaluation of the sensitivity, reliability and repeatability of infrared thermometry when compared to a rectal temperature probe which was used as a reference point. Two different strains of male rats, Wistar and DA of the same age (12 weeks of birth) were used in order to assess whether skin color affects measurements with the infrared thermometer. The temperature sites measured were abdominal area, head and lumbar region. Animals were anaesthetized with isoflurane via a face mask and afterwards shaved at the above stated areas. Repeated measurements were taken every 2 minutes from each site (5 measurements in total). From the statistical analysis appears that there is no difference ($P > 0.05$) between the temperature measurements using the two methods of thermometry in any of the areas mentioned above. In addition, skin color seems not to affect the measurements of body temperature by using the infrared thermometry. Furthermore, it was observed that when the rat was placed from a prone to a supine position, or the opposite, the temperature of the respective area immediately measured with the infrared thermometer, was increased due to the contact of the body with the surgical table. In conclusion, infrared thermometry could be used as a reliable method for the measurement of body temperature in rats, regardless of the strain.

P035 Physical and Chemical Composition of Urine of Rats and Hamsters

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The urinalysis is one of the most informative test in laboratory animals, and it is useful for knowing the health and physiologic status of animals. There is scattered information in the literature about the physical and chemical composition of the urine from rodents, and it would be useful to have reference values. The objective of this study was to define physical and chemical urine values for two rat strains and two hamster strains. We analyzed urine samples from Sprague–Dawley rats ($n = 26$), BD-IX rats ($n = 13$), Golden Syrian hamsters ($n = 15$), and GASH:Sal hamsters ($n = 15$). Commercial metabolism cages were used for urine collection. For chemical urinalyses, the commercial test was ten-patch test strip, and the urinary sediment was analyzed by optical microscopy. The appearance of the hamster urine was a white creamy paste, which contained amorphous calcium phosphate (ACP), while the rats showed pale yellow urine. In all strains except for BD-IX, the urine from male animals showed higher pH than female (8.2 ± 0.2 compared with 7.4 ± 0.3; mean ± SD). In addition, the urine from male hamsters had higher specific gravity and proteins than the male rats. In the urine sediment, hamsters exhibited an increased incidence of ACP, phosphate acid calcium crystals, oxalate calcium monohydrated crystals and total crystals. Thus, GSH and GASH hamsters might be useful for the study of alterations in which metabolism of calcium/phosphate is involved. All reference ranges of values of urine analyses will be detailed in the poster.

P036 Containment and Eradication Attempt of *Mycoplasma pulmonis* from Rat Colonies in a Conventional Animal Facility: A Case Report

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Four rat strains have been bred in the past years in our facility: Wistar, Sprague–Dawley, Hyperbilirubinemic GUNN, PVG C6 -/- (complement factor 6 deficient). All evidenced clinical signs related to *Mycoplasma* infection since 2009. Signs consisted in porphyrin staining, weight loss, dyspnea, rhinitis and exudate in the lungs. The infection, confirmed by PCR, led to infertility and nearly compromised the survival of the mutant colonies. The decision was taken to attempt *Mycoplasma* eradication. The Wistar and Sprague–Dawley strains were eliminated and a new colony was restarted with SPF founders. A small group of animals from the two mutant strains, commercially unavailable, was treated with antibiotics and used to restart the colonies. The Sprague–Dawley colony was kept in an IVC in a new room. The old rooms were sanitized and implemented with changing stations, washing corners, top-filtered cages and isopropyl alcohol-based disinfection procedures. The Wistar and the Sprague–Dawley, physically separated from the other colonies, have not evidenced mycoplasmosis since inception, in July 2011. Of the two mutant strains, kept in the same room, the PVG colony still appears Mycoplasma-free, while it reappeared in the GUNN colony: apparently the antibiotic treatment was effective in one strain and not in the other, which might be also related to a different sensitivity to *Mycoplasma*. In conclusion the procedures avoid *Mycoplasma* spreading outside the infected room and seem effective against *Mycoplasma* contagion from the positive colony to the negative one inside the infected room.

P037 Differences in the Diagnosis of *Helicobacter* spp. and Nematodes in Mice Using Animals from the Colony or Sentinels

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Health monitoring of the mice colony housed in the CNB animal facility, with 4500 cages, was performed as follows: from 2005 to 2008 animals from the colony were randomly selected for testing, and from 2009 through 2012 tests were performed with sentinels (CD1 females) placed in 50 open cages with dirty bedding added. During these years the colony has been positive continuously for *Helicobacter* and sporadically for nematodes. Comparing both approaches, there was a higher percentage (54.1%) of *Helicobacter* spp. positive animals when mice from the colony were tested, compared to the (21.5%) of positives when sentinels were used. Likewise, both approaches were used to perform parasitological tests on animals from other colonies arriving to the quarantine in order to avoid introducing nematodes in our colony. In those shipments were at least one of the arriving animals tested positive,

sentinels were placed in the quarantine. After 3 weeks all animals were tested and we found that the percentage of positives among arriving animals was higher (85.7%) than the percentage of positives found among sentinels (28.6%) Both results demonstrate that the diagnosis sensibility, to test *Helicobacter* spp. and nematodes in mice, is increased when animals from the colony are used, compared to sentinels. It also indicates that the use of sentinels might not only lead, in some cases, to false negatives, but it may also demand a higher number of animals tested in order to increase health monitoring accuracy in rodent colonies.

P038 What is Rodent Pneumonic CAR bacillus? From Full Genome Sequencing

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The cilia-associated respiratory bacillus (CARB), an unclassified, extracellular, gram-negative filamentous bacterium, colonizes the ciliated respiratory epithelium of rodents and causes persistent respiratory diseases. CARB was reported to grow in mammalian cell culture. Recently, we developed support cell free, CARB single culture system by using Vero E6 culture supernatants. Using this single culture system, we extracted genomic DNA and determined its sequences. [Materials and Methods] SMR strain of CARB isolated from rat was cultured in Vero E6 cell culture supernatants in nonadhesive plate. Growth of CARB was monitored using phase contrast microscopy. Grown CARB was confirmed by immunofluorescence assay and PCR. *Mycoplasma* spp. and other bacterial contamination were not detected. Collected CARB by centrifugation was treated by proteinase K and RNAse, and genomic DNA was extracted by phenol/chloroform method. Draft sequencing was performed using Roche 454 GS FLX+. [Results and Discussion] CARB genome sequence was about 1.44 Mbp long from 5 scaffolds contig and it did not possess plasmids. Open reading frames were extracted by MetaGeneAnnotator program analysis. BLAST search was carried out against nr databases. We found 34 tRNAs, one rRNA set and about 1,700 coding sequences (CDS). Then, we did further PCR sequencing and determined full genome sequences. CARB is known to belong to cytophaga-flavobacteria-bacteroides phylum by 16S rRNA gene sequences analysis. However almost CDS obtained from this study showed low homologies against reported genes indicating that CARB had quite unique genomes among bacteria whose genomes were analyzed to date.

P039 Impact of Fenbendazole on Neurobiologic and Metabolic Functions in Mice

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Fenbendazole is a broad spectrum benzimidazole anthelmintic used against gastrointestinal parasites. It is often used in experimental housing facility to treat rodents contaminated with pinworms. However, not much is known about its side effects, impacting on experimental data from rodents that underwent such treatment. The aim of this study was to measure the impact of fenbendazole on cognitive and metabolic functions in mice. In such a context, C57BL6/J mice were fed with diets containing or not fenbendazole. After 7 weeks of fenbendazole exposure, a group of mice were tested in a neurobiological pipeline including Morris Water Maze, Open Field, Elevated Plus Maze, Fear Conditioning Test, Endurance treadmill and Home cage activity monitoring. Separately, a second group of mice were tested in a metabolic pipeline including body weight measurement, food intake measurement, body composition, glucose tolerance test, adaptive thermogenesis test, energy expenditure measurement and noninvasive blood pressure measurement. In addition, blood samples were collected at different time points and hematology and biochemistry analysis were performed. Surprisingly, no major significant differences were observed in any of these neurobiological and metabolic analyses after fenbendazole exposure. Therefore the present study suggests that in a context of scientific experiments, fenbendazole treatment could be recommended to treat animals with pinworms, because it seems to have no major impacts on neurobiological and metabolic functions in mice.

P040 Occurrence of Rodent Parvoviruses in Mouse and Rat Colonies from 17 Brazilian Breeding Facilities

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Rodent parvoviruses are challenging pathogens to eradicate from laboratory animal facilities. The aim of this study was to evaluate the occurrence of Minute virus of mice (MVM), Kilhan rat virus (KRV), H-1 parvovirus (H-1), Mouse parvovirus -1 (MPV-1); Rat minute virus -1 (RMV-1) and Rat parvovirus 1 (RPV-1) in seventeen breeding facilities of public institutions from different geographic regions of the Brazil. IFA, IHA and PCR were used for diagnosis of parvoviruses in 563 mice and 167 rats. The occurrence of murine parvoviruses identified by serology was 18.3% (MVM - 6.2%; MPV - 12.3%) and the positivity ranged from 0 to 22.5% in different regions. When tested by PCR the parvoviruses occurrence was: 49.2% (MVM - 12.3%; MPV - 43.5%) and positivity ranged from 16.7 to 100.0%. The occurrence of rat parvoviruses by serological methods was: 40.7% (H-1 - 1.8%; KRV - 3.0%; RPV-1/RMV-1 - 35.9%) and positivity were detected only in the Southeast region (51.5%). PCR testing showed rat parvoviruses occurrence in 73.7% (H-1 - 0%; KRV - 6.0%; RMV-1 - 37.7%; RPV-1 - 54.5%) and positivity ranged from 25.0 to 100.0%. The highest occurrence of parvovirus infection (35% to 100%) was observed in institutions of the State of Minas Gerais where co-infections: MVM and MPV-1; RPV-1 and RMV-1 were identified. Overall, MPV-1 and RPV-1 were the most frequently detected viral species in all regions. Noteworthy, the vast majority of the animal breeding facilities evaluated in this study did not present appropriate protective barriers to prevent pathogen transmission.

P041 Measuring Health Status in the Cephalopod Mollusc *Octopus vulgaris*: Use of an Artificial Crab to Measure Predatory Responses

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The inclusion of cephalopods in Directive 2010/63/EU will require the development of objective methods to assess their welfare on daily basis in the laboratory. The latency of *Octopus vulgaris* to attack a crab (*Carcinus maenas*) presented in the tank has frequently been used as an indicator of overall health. However, this procedure requires the use of a live crab withdrawn before it is seized, and which may be used repeatedly. To assess the potential for replacing the use of a live crab we have compared the latency of a group of thirty *O. vulgaris* (200-500 g, body weight) to attack a live crab with that to attack a freshly killed crab and an artificial peeler crab (Berkley Gulp Peeler Crab). A mixed subject design was used with each challenge presented on five occasions on consecutive days. Latencies were compared using Wilcoxon-matched –pairs signed rank tests. All animals attacked the artificial crab, the live

and dead crabs. There was no difference in the latency to attack the dead crab as compared to the live crab, but the latency to attack the artificial crab was longer than to attack the live crab ($P = 0.008$). This pilot study shows that an artificial crab could replace a live crab assess the willingness to attack as an index of overall health in the octopus. Thus an artificial crab may be utilized as alternative method to evaluate latency values to assess octopus' normal predatory behavior.

P042 Frequent and Rare Clinical Findings in Rodent Animal Facilities

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Rodent diseases can have a devastating effect on the experimental results or compromise the sanitary qualification of the animal facility. Maintain a correct health status of the housed rodents is one of the main tasks of the Veterinary Area of an animal facility. Well-designed health monitoring programs and properly trained staff are two basic tools to achieve this goal. Based on our animal facility casuistry we have developed a collection of observed cases along last year. The aim of this review consists in exposing some of them according to two criteria: the most common diseases, and in contrast, the rarest ones. On the one hand, we have the ulcerative dermatitis; very typical of C57BL/6 strain, which begins with a simple scratch of the skin and ends in a very serious bacterial infection and ulceration. Other frequently found diseases are malocclusion, rectal prolapse, blepharitis, conjunctivitis and overgrown tumors. On the other hand, we report one case of ringtail, consisting in circular constraints on the appearance of the skin of the animal tail. Other uncommon founds reviewed are the situs incompared with, a congenital disorder in which the major visceral organs are reversed or mirrored from their normal positions; sialodacrioadenitis in rats; and mouse general subcutaneous aedema. This review intends to give additional tools for users of experimental animals to improve their skills in detecting clinical signs and diseases, which will support in the maintenance of the optimal health status of animal facilities.

P043 Global Animal Welfare Standards Inside and Outside Our Walls

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Pfizer has a long standing commitment to high standards of animal welfare emphasized in its publicly available policy. This commitment applies wherever research is carried out, inside or outside the company. Two important policy decisions contribute to these standards. All Pfizer sites will be AAAALAC-accredited and all proposals for external work must be reviewed. An Accreditation Readiness Team assists Pfizer sites in preparing their AAALAC program description and then carries out a pre-accreditation site visit. These visits are important in ensuring sites meet Pfizer policy and AAALAC standards; that the Program Description is well written and that sites have robust processes in place. In 2012 five (5) sites were approved for first time accreditation or re-accreditation. For studies with external partners all proposals from all divisions of Pfizer Inc must be reviewed by our Risk Assessment team. We have adopted a risk based approach that provides adequate review of the CRO's (or collaborators') animal care and use program. A short questionnaire completed by the proposer and the external site allows us to evaluate the quality of animal care and institutional processes. Based on the responses the site will be assigned high, medium or low risk and a decision on the need for an animal welfare site audit made. Some important factors in determining risk are species proposed, type of procedures and experience of the external partner. In 2012 we carried out 205 risk assessments, and 15 audits.

P044 Withdrawn

P045 Retrospective Comparison of Reproductive Success of F0 and F1 *Macaca fascicularis* Populations

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Our institution is a *Macaca fascicularis* breeding center established in Mauritius since 1990. The breeding pool, originally made of F0 monkeys, has progressed towards a close colony system with both F0 and F1 breeders. The reproductive, demographic and clinical information stored in the database since 20 years allows us to perform valuable retrospective investigations on large populations of group housed macaques. The growing interest of the scientific community in the replacement of F0 breeders by captive-bred breeders raises questions on the feasibility and the zootechnical implications of this shift. This analysis, based on over 30,000 births, focused on the reproductive success of the breeders and the comparison between the F0 and F1 populations. We measured the population's reproductive success through the analysis of the birth rate, the pregnancy outcome and of some indicators of their mothering ability. Captive bred females (F1) showed a lower birth rate and a higher incidence of obesity than wild caught females (F0). Primiparous breeders had a significantly lower infant survival rate, this being more pronounced in the F1 population. Infant survival rate improved as females' parity increased with F0 females remaining more successful than F1 females. The analysis of infant necropsy findings revealed that mismothering was the main cause of infant mortality for both F0 and F1 females. Although repeated experience with pregnancy and aging of the females play a key role in the successful rearing of infants, an uneven learning process of maternal behavior may explain the differences between the two populations.

P046 Schematic Comparison, Legislative and Functional Committees Associated with the Use Animals in Research in Latin America (Study Review)

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Basic, medical and biomedical areas of research use animal models to explain phenomena of interest, which in turn requires the generation of new methodological schemes, working groups, normativities and legislative strategies that ensure welfare conditions for the animals used in research. In Latin America, however, the absence and/or ineffectiveness of laws for the welfare of animals used in research is reflected in a limited number of institutional committees with clear guidelines and infrastructure. Thus arise the need to reinforce IACUC's and promote their creation as instruments for institutionalizing everything related to the Care and Use of Animals with prerogatives to discuss and propose policies related to these topics with institutional authorities. The aim of this work is compare laws, regulations, roles and members of ethics committees associated with animal experimentation in Latin America. While in countries like Brazil, Mexico and Uruguay punitive and regulatory laws for animals in research are defined by the Congress, in other countries like Costa Rica, Cuba and Colombia, the regulation and punishment is assumed by academic entities (which may or may not have IACUC's) or are defined in standards including specific laws to animal welfare. This review illustrates a broad weakness in the development of better tools to protect the health and welfare of animals used in research.

P047 Protocol Review in Compliance with AAALAC and EU Directive 2010/63/UE

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In compliance with regional legislation, an oversight body was created at our institution in 2001. The main function of the body, initially, was to review the protocols, but in 2006 the oversight body became a IACUC (Institutional Animal Care and Use Committee) in compliance with AAALAC accreditation new requirements. The IACUC is in charge of review the animal and use program, animal procedures, performing the Post Approval Monitoring (all the ongoing procedures once a year), adapting the composition of the committee and inspecting the facilities. In 2012, in compliance with new European directive, retrospective assessment was included as a new IACUC responsibility (all procedures are assessed every two years, before renewal). In the last 12 years, 300 procedures were evaluated (since 2008, annual average of 45). Half of the procedures reviewed belong to the field of oncology, 40% correspond to genetic models of human diseases and 10% are animal facility procedures. In our Institution, 72% of all procedures have required authorization from competent authority whereas the rest, have been approved by a simplified administrative procedure. Since 2006, the most relevant inconsistencies found in the Post Approval Monitoring have been the no compliance with the endponits (50%), the analgesia and anesthesia (20%), the product administration (20%) and the method of euthanasia (10%). By the performance of a retrospective assessment (25 procedures) an absence of the review procedure goals and few implementation of the 3Rs were detected. Compliance with Directive 2010/63/EU and its implementation before its transposition has been easier being an AAAALAC-accredited Unit.

P048 Laboratory Animal Science: From Bench to File Drawer

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Publication bias, the tendency to publish significant results and not publish negative ones, is a serious concern to the ethical conduct of research involving both animals and people. In translational stroke research, a systematic review of stroke models identified a nonpublication rate of 14% leading to a 30% overestimate of the efficacy for the treatments. Such biased reporting has negative impacts on the welfare of the end beneficiaries of research: participants in clinical trials and patients awaiting treatments. However, animals that are used in experiments which are never published, and thus never utilized by the research community, may also have their welfare compromised for no benefit. Here, we review how the clinical research community has dealt with publication bias and outline how those working with in vivo animal models in the pre-clinical community can approach the same challenge. One of the surest ways to achieve better science with fewer animals is to ensure that the animals used in research laboratories are fully utilized; including the publication of research findings.

P049 Using Synthesis of Evidence to Advance the 3Rs Principles in Science: Better Science with Fewer Animals

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At the eighth World Congress on Alternatives and Animal Use in the Life Sciences, August 21 to 25, 2011, Montréal, Canada, the Montréal Declaration on the Synthesis of Evidence to Advance the 3Rs Principles in Science was unanimously proclaimed by delegates. The 3Rs principles are incorporated into guidelines, regulations and legislation worldwide, including EU Directive 2010/63, and are seen as fundamental to the ethic of animal experimentation. There is universal agreement that animals should only be used for scientific purposes when no appropriate Replacement alternative is available, and only when scientifically and ethically justified. There is also agreement that animal studies should be of high quality and relevance, so that animals' lives are not wasted. In a systematic review (one form of synthesis of scientific evidence), all relevant studies addressing a specific research question are identified, appraised and selected following pre-defined criteria. Hence, the Montréal Declaration, in calling for a change in the culture of planning, executing, reporting, reviewing and translating animal research, to support synthesis of evidence is aimed at supporting better science. Signees to the Declaration agreed that encouraging synthesis of evidence should help to: 1) identify areas where additional animal studies may not be needed; 2) inform the ethical review of animal studies; 3) improve scientific reporting; and 4) improve the value of animal research models. In the long term, this should achieve the principles of the 3Rs – better science with fewer animals.

P050 Brief Overview of the Protection of Experimental Animals in Slovenia

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In Slovenia the field of the protection of experimental animals has actually started to regulate with the adoption of the Animal Protection Act and other subsidiary legal acts that defined legitimate purposes and conditions on which animals for experimental purposes may be used. They came into force in 2004 and were based on Directive 86/609/EEC, which has started to regulate the legislation on the protection of experimental animals in Europe. Since 2004 a significant amount of work has been performed in Slovenia on the field of the protection, care and use of live animals in scientific research. In 2005 Ethical Committee for laboratory animals of the Republic of Slovenia was established. From 2007 on courses on laboratory animal science have been organized for persons working with experimental animals. With the adoption of the Directive 2010/63/EU in 2010, Slovenian Society for Laboratory Animals (SSLA) has been restored to life. Although the SSLA was created in 2001, the first activities of the SSLA started in November 2010, when the SSLA (in collaboration with the Ethical Committee and the Committee of researchers) has taken important part in the process of implementation of the Directive 2010/63/EU. In January 2013 the 1st Congress of SSLA was organized with the purpose to bring together all Slovenian organizations and companies that are using experimental animals and to gain insight into their current work, techniques and the equipment they use and possess and to introduce the Directive 2010/63/EU and the changes it brings to the Slovenian legislation.

P051 End-User Experiences of a New Legal Framework in Laboratory Animal Science: Pitfalls and Learning Opportunities

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At the start of 2013, the Irish Medicines Board became the competent authority responsible for the implementation of Directive 2010/63/EU in the Republic of Ireland, taking over responsibility from the Department of Health. The consequences of this change for research institutions include an increase in record keeping regarding, amongst others, animal use, staff training and researcher training. This change coincided with the opening of a new purpose-built multiunit facility at our institution and the piloting of a new electronic data management system. We describe the experiences at our institution since these changes, interactions with the new authority, and the changes in record keeping and data management deemed necessary. The benefits and drawbacks of designing and building a unit at a time of regulatory change are discussed. We discuss the implementation of the electronic data management system and its effect on record keeping, breeding performance and regulatory compliance. The influence of regulatory change on the design and implementation of data management is explored. We evaluate the effect of these changes on animal welfare, staff morale and wellbeing, and the quality of research at both the existing and new units. Unforeseen consequences of the regulatory changes on various aspects of the running of the unit, such as the breeding of transgenic lines, genotyping and the regulation of euthanasia, are explored.

P053 Comparative Pharmacokinetics of Ketamine and Xylazine in Old and Young Sprague–Dawley Rats

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The main objective of this study was to compare the pharmacokinetics of ketamine and xylazine in young (8 to 12 weeks) and old Sprague–Dawley (2 to 2.4 y) rats when administered at an anesthetic dose. Rats (n = 6/group) received intraperitoneally 125 mg/kg of ketamine and 10 mg/kg of xylazine). Selected time points for blood collections were 15, 30 min and 1, 2, and 4 h post ketamine-xylazine administration. All samples were analyzed by high performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS). In young and old rats, the withdrawal reflex was absent during ketamine-xylazine anesthesia and was present at respectively 1.1 (± 0.2) and 2.6 (± 0.7) h following the drug combination administration ($P < 0.0001$). The first voluntary movement following ketamine-xylazine anesthesia was observed at 1.5 ± 0.2 and 4.9 ± 1.0 h in young and old rats respectively ($P < 0.0001$). In old rats, drug availability (AUC) was 6.0 and 6.7 times greater for ketamine and xylazine respectively, when compared to young rats ($P < 0.0001$). For both drugs, the rate constant of elimination was greatly decreased (ketamine 0.55 compared with 0.08; xylazine 0.53 compared with 0.05) and the elimination half-life was significantly greater (ketamine 8.5 compared with 1.3; xylazine 12.9 compared with 1.3) in old rats ($P < 0.0001$). In conclusion, age has a significant effect on ketamine and xylazine plasmatic concentrations, changing significantly the pharmacokinetics of these drugs, which translates into longer anesthesia duration and recovery times with aging.

P054 Preference of Group-Housed Laboratory Mice Regarding Different Bedding Volumes

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In order to ameliorate the husbandry of laboratory mice, a number of different environmental improvements according to the behavior of mice (such as climbing, nesting and burrowing) have been developed. Even though a large amount of bedding could be considered as an improvement of the environment, very few studies have focused on the depths of bedding. Therefore, the aim of this study was to understand the preference of group-housed laboratory mice regarding three different bedding volumes (0.5 L, 1.5 L or 6 L per Type III cage). The bedding volumes were tested in pairs using two inbred strains of female mice (BALB/c and C57BL/6). After arrival, at six weeks of age, the mice were divided into groups of four, followed by a 2-wk adaptation period. The automatic system applied for preference testing consists of two type III cages connected by a Perspex tube. During the entire experimental setup the cages on both sides contained food, water and the same type of bedding, coarse-grained aspen chips. With the help of subcutaneous transponders carried by each mouse and two sensors at the end of the tube, the system is able to register the location of each animal within the group. The dwelling time, as well as the crossing activity of every mouse can therefore be calculated individually for further analysis. According to the validation (98% in accordance with video analysis) this system is able to provide reliable results.

P055 Environmental Enrichment Does Not Affect Nociceptive Behaviors in Neuropathic Rats but Affects Exploratory and Anxiety Behaviors

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We have previously shown that neuropathy and depression in mice with long standing neuropathic can be reversed with environmental enrichment. In this study, we investigated the impact of environmental enrichment in rats up to three months after chronic nerve injury. Animals (n = 24, 7 to 8 wk, SD Sprague–Dawley rats were housed (2/group) in either standard polycarbonate cages or rat enrichment cages. Following one week of acclimation, some rats (n = 12) underwent chronic constriction nerve injury (CCI) surgery to the right sciatic nerve while under general anesthesia to produce a chronic neuropathic pain model. Other animals (n = 12) underwent a sham surgery. Animals were the evaluated prior to, and once a month for 3 months on different behavioral tests (comprised mechanical (von Frey filaments) and heat sensitivity (Hargreave's test) as well as exploration (open field) and anxiety-like (elevated plus maze)). One month following the surgery, half of the rats in each group were either left in the standard rats cages or placed in the double Decker cages. Results environmental enrichment didn't affect mechanical or heat sensitivity however exploratory and anxiety-like behaviors were significantly improved ($P < 0.01$). These results clearly show that environmental enrichment can have a significant impact on exploratory and anxiety-like behavior in rats but does not affect pain hypersensitivity in a neuropathic pain model. Importantly, cage size would appear to preserve higher brain functions without affecting chronic pain processing in rats.

P056 Effects of Housing Condition on Reproductive Parameters in a Baboon Colony

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In the past several studies have examined stress induced by removing individuals from their social groups, and found that moving to individual housing represents a significant stress for many primates. In the present work we report the results of comparing the reproductive performance of a colony maintained in two very different living conditions. The birth records and infant survival of a group of baboons under different housing condition were examined between 2001 and 2010 to determine several reproductive parameters. A group of sixteen female and four male baboons (*Papio* spp.) aged 6 to 12 years. The reproductive events (effective copulas, alive born, death born, abortions and newborn viability at 1st week, 3rd month and 1st year from birthday) were recorded during 5 and 5 years in the indoor and outdoor social cages housing, respectively, and compared. The results indicate that pregnancy is accelerated in outdoor conditions, while postpartum amenorrhea and interbirth interval are reduced. Interbirth interval in outdoor facilities was shorter. The stress of extended individual housing significantly reduced negatively fecundity. The shorter interbirths interval may represent the lower physiologic limit, and may be explained by lower stress in the baboons housed outdoor and an earlier weaning age for infants. Data presented in this report provide a comprehensive profile of reproductive parameters for laboratory-housed baboons, which may aid other facilities in the reproductive performances assessment of captive baboons. The higher percentage of newborn surviving at the recorded periods suggests the benefits of the enriched environment in outdoors facilities.

P057 Animal Welfare Aspects of different Bleeding Methods in Mice

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According to Article 13 of the new EU-Directive 2010/63/EU having the choice of different suitable methods, the method, which causes least pain, suffering and harm, has to be chosen. In a primary experiment pain and suffering caused by a short time inhalation anesthesia was

studied. In the final experiment the following recommended (GV-SOLAS) bleeding methods in mice were studied in respect to pain, suffering and harm: 1) retrobulbar puncture with a hematocrite capillary, 2) retrobulbar puncture with a glucose capillary under anesthesia, 3) puncture of the tail vein, 4) puncture of the Vena saphena, 5) puncture of the Vena facialis, 6) puncture of the subclavicular venous angle under anesthesiaFollowing the blood sampling the behavior was observed for 5 minutes in the home cage, after 15 minutes the corticosterone level was determined and then the behavior in an open field test was recorded for 5 minutes. Histologic samplings were examined 3 hours, 3 days and 14 days after blood sampling. The result of the first experiment showed, that all anesthesia studied caused. Any method of blood sampling led to an additional increase in corticosterone concentration in the following order (low to high): retrobulbar puncture with hematocrite capillary, retrobulbar puncture with glucose capillary, puncture of the Vena saphena, puncture of the tail vein, puncture of the Vena facialis, puncture of the Vena facialis. Histologic changes were found following all blood sampling methods.

P058 Withdrawn

P059 Multilevel Caging Enhances the Welfare of Rats as Assessed by a Spatial Cognitive Bias Assay

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To evaluate welfare of rats housed in a commercially available multilevel caging system, 36 male Sprague–Dawley rats were randomly assigned as follows: (1) bottom level only, no change; (2) access all levels, no change; (3) bottom level only, then access all levels; or (4) access all levels, then bottom level only. They were trained to perform a spatial discrimination cognitive bias task; the housing environment was changed (per treatment group); and they were subsequently tested with three ambiguousprobelocations (reward plus, neutral, or nonreward plus). The latency times to approach locations were compared between groups (ANOVA). Findings were consistent across locations, but only average latency data for the neutral location is presented here. Group 3 average latency time was significantly decreased (15.51 sec ± 5.73 sec, $P = 0.0375$), suggesting positive cognitive bias associated with enhanced caging access. Group 4 average latency time was significantly increased (37.03 ± 5.65 sec, $P = 0.0252$), suggesting negative cognitive bias when enhanced caging access was removed. There were no differences in the average latency times in groups 1 and 2, where there were no changes in their environment (22.03 sec ± 5.65 sec and 29.11 sec ± 5.73 sec, respectively, $P = 0.5202$), suggesting habituation. Increases in neutrophil:lymphocyte ratio have been associated with chronic distress. A significant decrease in this ratio was seen in group 2 as compared to the other groups ($P = 0.0426$), suggesting improved welfare. These findings suggest the welfare of these rats was enhanced through access to both levels of multilevel caging.

P060 Evaluation of Wellbeing of Rats Housed in Commercially Available Multilevel Caging with Variable Use of Red-Tinted Polysulfone

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Current laboratory conditions house rats in clear caging in brightly lit rooms. As their natural history suggests a preference for low-light conditions, this practice is likely stressful for rats. The retinal anatomy of rats suggests that they are unable to see the red spectrum of light, so red-tinted caging would replicate a darkened condition for the rodent. This project focused on examining the welfare of rodents housed in red-tinted caging using a multilevel caging type that allowed the rat to select their microenvironment. Thirty-four Sprague–Dawley rats were divided into five treatment groups: entirely clear, entirely red, red top/clear bottom, clear top/red bottom, and entirely clear with a red intra cage shelter. Rodents were allowed to acclimate to their housing treatment for five weeks before being tested in the elevated plus maze and open field test. Passive video was collected to determine use of the given environment and the role of color in selection of microenvironment. Results from the passive video analysis indicated that rats actively sought the red environment when it was provided. Rats within the all red caging showed increased exploratory behavior in the open field apparatus, correlating with increased use of space within the cage, while those in a clear cage with a red intra-cage shelter exhibited increased anxiety behavior in the elevated plus maze. These findings suggest that an all red cage could be beneficial to the rodent's wellbeing while intracage shelters can be detrimental.

P061 How Do C57 and BALB Mice React to a Mouse House in Their Home Cages: Encouraging Results for Researchers

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Animal welfare depends on the possibility to express specie-specific behaviors and can be strongly compromised in socially and environmentally deproved conditions. Nesting material and refuges are very important resources to express these behaviors and should be considered as housing supplementation items rather than "environmental enrichment". We evaluated the effects of housing supplementation in standard settings in laboratory mice C57BL6/JOlaHsd (C57) and BALB/OlaHsd (BALB) young male and female mice. Upon arrival they were housed in groups of four in standard laboratory cages and after 10 days of acclimatization, a red trasparent plastic triangular-shaped Mouse House™ was introduced in half of the home cages. No additional nest material was provided. Animals with mouse house (experimental) and animals without nouse house (control) were observed in different contexts for subsequent 36 days. Body weigth and food intake, home cage behaviors, emotionality and response to standard cage changing procedures were measured. The presence of a mouse house in the home cage did not interfere with main developmental and behavioral parameters or emotionality of BALB and C57 male and female mice. Additionally, both strains habituated to the house in about a week, but made use of it differently: BALB mice interacted and used more the house compared to the C57 strain. Our results suggest that mice habituated to the mouse house in one week and its presence did not affect the behaviors. Researches can thus be encouraged in using mouse houses, also in view of the implementation of the new EU Directive (2010/63/EU).

P062 Withdrawn

P063 Just Give Me Some Buprenorphine with My Pellet! A Double Blind Clinical Trial in Mice

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Postsurgical self-administration of analgesics in rodents is an interesting technique to avoid further manipulations in operated animals. There are several techniques described using gelatine, nutella and even honey, but all of them require some habituation for the animals to reach a good intake, because those food are not included in a normal rodent diet. This greatly complicates planning of experiments. In this study we evaluate the impact in welfare of buprenorphine mixed with a standar lab animal diet (2919 Teckland-Harlan). Twenty Hsd:ICR pregnant mice (E11-12; Harlan) included in several other studies, were submitted to Intrautero electroporation. They were anesthetized with isofluorane

and all of them received buprenorphine sc before surgery (0.1 mg/kg). The buprenorphine group (12 mice) received only one pellet mixed with buprenorphine dissolved in 1 ml of glucosaline (0.03mg/ml). The placebo group received a pellet mixed just with glucosaline. The animals were individually housed. A person, who was blinded to the treatment, evaluated the next day, the following items related to animal welfare: facial expression, according to the Mouse Grimace Scale (MGS), food intake and weight. The mice included in the buprenorphine group had greater food intake, median (p25 to p75): 2 (0.4 to 3.3) compared with 0.4 (0.4 to 0.9) g, $P < 0.05$, loosed less weigth: 2.9 (1.2 to 6.4) compared with 5.7 (3.8 to 6.8) g, $P = 0.05$ and had minor MGS values: 0 (0 to 0.4) compared with 0.4 (0.2 to 0.8) points, $P = 0.3$. Those data suggested that buprenorphine made mice feel better and had a beneficial impact in their evolution.

P064 Assessment of Laboratory Mouse Welfare Using Animal-Based Measures

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Protocols to assess laboratory animal health and welfare are commonly used in experimental studies to record the outcome on the animal as a result of an experimental input. In laboratory mice, many of the welfare problems do however occur in the home cage and are irrespective of the type of research. Assessment of the home cage environment is commonly performed using resource-based measures for example, group size or access to nesting material. Since animals react differently to the same environment depending on age, sex, genetic background, previous experiences etc., it is more relevant to assess welfare as animal-based (outcome) measures rather than resource and management-based (input) measures. The same animal-based measures can then be applied in different types of animal facilities, allowing comparisons between them, or to track changes over time following modification in housing and management within the same facility. The aim of this project was to design and test a protocol for assessing the welfare of laboratory mice in their home cage using only animal-based measures. The parameters used were selected to cover different aspects of animal welfare, namely good feeding, housing and health, and appropriate behavior. These aspects are divided into 12 welfare criteria and parameters were selected to match the criteria, to be feasible in practise and, if possible, be already validated indicators of mouse welfare. The protocol was tested at three different animal facilities in Sweden during 2012. The development of the protocol and results from the testing will be presented.

P065 Comparison of Inhaled Isoflurane and Topical Anesthetics for Tail Biopsy in Preweanling Mice

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Tail biopsy of laboratory mice for genotyping purposes has been extensively studied to develop refinements for this common procedure. Our prior work assessed tail vertebral development in differing mouse strains (aged 3 to 42 d) and analyzed behavior and activity in mice (aged 21 to 45 d) biopsied under isoflurane anesthesia. To better assess the impact of tail biopsy on pre-weanling mice, this study sought to compare the relative performance of inhaled or topical anesthetics on responses to the procedure. We evaluated BALB/cANNCrl mice ($n = 80$), aged 18 to 21 d, that underwent a 5mm or sham biopsy and were treated with inhaled isoflurane or with topical anesthetics (cetacaine or ethyl chloride). Control animals received no anesthetic intervention. Mice were observed at the time of biopsy and up to 60 minutes for behavioral changes to determine an acute observation score. Locomotor activity was recorded post-biopsy for 120 minutes and histologic examination was conducted on tail sections at day 0, 1, and 7 post-procedure. Biopsied mice had significantly increased acute observation scores at 10 minutes post-biopsy and had significantly decreased activity, regardless of type of anesthetic ($P < 0.05$). Application of ethyl chloride significantly increased acute observation scores at 10 minutes post-biopsy compared to mice that received isoflurane or no anesthesia ($P = 0.01$). Based upon histology reviews, inflammatory changes in tail tissues remained elevated up to 7 days post-biopsy and were influenced by specific anesthetics. Our experimental paradigm indicated that neither inhaled isoflurane nor topical anesthetic appreciably enhanced wellbeing for mice at pre-weanling ages over control mice biopsied without any interventional treatment.

P066 Single-Use Instrument for Contamination-Free Simultaneous Ear Marking and Genotyping of Rodents

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Genetically modified mice need to be genotyped and identified. They are often identified by ear punching, but for genotyping the tip of the tail is clipped off. From the animal welfare perspective it would be preferable to use the tissue released by ear punching also for genotyping and avoid extra tail clipping. A major drawback of using the ear punch material is DNA cross-contamination from mouse to mouse because re-usable clippers are in use. Another drawback is the risk of pathogen transmission. Our idea was to develop a single-use disposable device that allows simultaneous clipping and sampling of ear tissue into a standard tube for downstream processing, for example, PCR. Prototypes have been developed together with an industrial partner and tested. Using such a device, millions of painful procedures might be avoided per year. This would be a major contribution to the 3Rs.

P067 Improvement of Experimental Conditions in a Model of Neuropathic Pain in the Rat

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With respect to our preclinical research activities, improvement in animal welfare is one of our main objectives. As part of our strategy to improve the experimental conditions in models of neuropathic pain, we planned to improve our surgical procedures (in particular for reducing pain and suffering) and to include environmental enrichment during the housing of the animals. However, these modifications may have an impact on the behavior of animals which could affect the results obtained. Among our validated models of neuropathic pain, the Chung model (spinal nerve ligation) was selected to study the impact of the two procedural modifications. On arrival in our facilities and until the end of the study, 20 rats were placed in nonenriched housing conditions and 20 rats were placed in enriched housing conditions. Half of the rats (10 without and 10 with environmental enrichment) were operated according to our validated surgical procedure whereas the other rats were subjected to improved surgical conditions. At least 2 weeks after surgery, rats were evaluated consecutively for tactile and thermal allodynia and hyperalgesia. Regardless of the surgical conditions, the results from the different behavioral tests were consistent and comparable to our historical data; however, the use of enrichment tended to slightly increase the variability. Based on these conclusions, improved surgical conditions could be applied without impact on our validated model, a change in environmental conditions is recommended but should be reduced to a limited level of enrichment (for example,, nesting material) to maintain reliability of the model.

P068 Impact of Superovulation and Mating on Juvenile C57BL/6 Mice

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Embryos of C57BL/6 mice are widely used. Because of the negative impact of sexual maturation on the number of superovulated oocytes, prepubescent B6 females are preferentially used. Here we provide results to estimate the degree of distress caused by superovulation and mating of juvenile and adult mice. Two groups of 22 C57BL/6NCrl mice (3 to 4 wk compared with 7 to 8 wk) were separated at delivery into single cages. Feces were collected daily starting from the day of arrival. Out of 22 mice per group, there were 6 control mice which were neither treated nor mated. On day 6 after arrival, the remaining 16 mice per group were superovulated and mated with males for 2 hours. The mating behavior was assessed. Next day, the mice were checked for a vaginal plug. After flushing the oviduct ova and zygotes were counted. The vagina was excised for further histologic examination. As expected, the number of zygotes was significantly higher in juveniles as compared to adults. Observation of mating behavior showed that reluctant adult females tended to fight the male's approach, whereas juveniles preferred to take flight. The level of glucocorticoid metabolites (an indicator for distress) was not different between the two age groups. Histologic investigations showed no vaginal injury or lesion in either group. Therefore, our results reveal no animal welfare problem with using juvenile mice for superovulation and mating. Considering the higher yield of zygotes, it is therefore advisable for C57BL/6 to use prepubescent mice in order to reduce the number of required donor animals.

P069 How Expert Systems Can Help to Ease the Suffering of Animals

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The EU-Directive demands a permanent and topical stress monitoring of animals in an experiment in order to minimize the stress as early as possible and to document the situation. Expert knowledge about the expression of stress of animals is being collected with the goal to derive the proper actions to handle the situation. The technology of Expert Systems Software apply knowledge (rules) to facts (symptoms and base data) and derives actions (Services) based on the met conditions of the rules. The integration of such an Expert Systems (Software) with an Animal Management Information Systems can play an important role to diminish the suffering of animals in the daily routine work in an animal house even if the human expert is not immediately available at the location of the animal. Thus using an Expert System allows to take the suggested actions shortly after perceiving the stress symptoms of the animal. The talk discusses the basic technics of expert systems with some simple examples and shows the prerequisits of animal information systems to embed an expert systems in the dayly routine processes of an animal facility.

P070 The Analgesic Infusion of Lidocaine–Ketamine in Combination with Either Morphine or Fentanyl Does Not Provide a Clinically Relevant Reduction of the Minimum Alveolar Concentration of Sevoflurane In Pigs

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The aim of the study was to determine the effects of a constant rate of infusion of lidocaine and ketamine in combination with either morphine or fentanyl on the minimum alveolar concentration (MAC) of sevoflurane in pigs. Six healthy crossbred pigs were premedicated with ketamine and midazolam and anesthesia was induced and maintained with sevoflurane. Then pigs were administered ketamine (0.6 mg/kg/h) and lidocaine (3 mg/kg/h) combined with either morphine (0.24 mg/kg/h; MLK) or fentanyl (0.0045 mg/kg/h; FLK) and the control group received a Ringer's lactate solution. Doses were similar to those previously studied in dogs and producing a significant reduction of the MAC. Animals were monitored for heart rate, mean arterial blood pressure, oxygen saturation determined by pulse oximetry (Spo2) and body temperature. The MAC was determined with the application of a clamp on the dew claw as the standard noxious stimulus. The MAC value was obtained with the up and down method and determined twice. The first MAC was 1.8 ± 0.3%, 1.8 ± 0.3% and 1.6 ± 0.2% in the control, MLK and FLK groups, respectively. The second MAC determination was 2.1 ± 0.2%, 2.2 ± 0.5% and 2.0 ± 0.5% in the control, MLK and FLK groups, respectively. No differences between groups were determined ($P > 0.05$, one-way ANOVA). Differences observed between the first and second MAC determinations were probably due to the drugs employed for premedication. In conclusion, the administration of MLK or FLK in pigs do not provide a significant MAC reduction suggesting relevant differences when compared to other species such as dogs.

P071 The Effect of 2 Different Individually Ventilated Cage (IVC) Systems on Laboratory Mouse Behavior and Anxiety

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The environment in which a laboratory animal is housed can significantly influence its behavior and anxiety, acting as a potential confounding factor for those studies in which it is utilized. This study investigated the impact of two IVC (individually ventilated cage) housing systems on the behavior and anxiety of two common strains of laboratory mice. Subjects were juvenile female C57BLj and Balb/c mice (n = 128) housed in groups of four in two different IVC systems for seven weeks. System One had air delivery at the cage 'cover' level at 75 ACH (Air Changes/Hour) and System Two had air delivery at the 'animal' level at 50 ACH. Mice were assessed twice a week (for example, bodyweight) and at the end of the study (for example, anxiety tests). Our results showed significant differences in behavior and anxiety between strains and between housing systems. Mice in System One, regardless of strain, defecated less in the elevated plus maze (EPM) ($F_{1,28}$ =12.2, P = 0.002), spent more time in the open arms of the EPM ($F_{1,28}$ =18.5, P < 0.001), and more time in the central zone of the open field (OF) ($F_{1,28}$ =6.2, P = 0.019). Strain differences in anxiety were seen in the increased defecation by Balb/c mice in the OF ($F_{1,28}$ =60.2, P < 0.001) and EPM ($F_{1,28}$ =19.6, P < 0.001) and less time spent in the open arms of the EPM ($F_{1,28}$ =17.2, P < 0.001) compared to C57BLj mice. These results suggest that System Two (air entry at the 'Animal' level at 50 ACH) was more anxiety inducing for mice of both strains, which could impact upon experimental data.

P072 Canine Behavioral Management and Socialization in a Research Facility

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Providing social interactions for dogs is considered a highly valued enrichment, but forming and maintaining compatible groups of dogs can be challenging. Establishing, breaking up and reforming groups of dogs frequently occurs in a research setting. This can potentially cause distress and injury. Understanding canine social structure and effectively managing social groups of dogs is an important component of their welfare. We have developed behavioral assessment, socialization and husbandry procedures to enhance our canine care and welfare program.

Our dogs are generally housed together in groups of 3 to 6 and exercised together in groups of 6 to 12. Observing individual behaviors and group dynamics are critical in deciding which dogs to place together. A key component of the program is identifying each dog's rank within the group. Our goal is to have one clear dominant dog as the Alpha with one or more Beta and/or Omega companions in each group. We have developed a behavioral assessment process to identify and place dogs in harmonious groups. Initial social introductions are done in a neutral area. Changing our caging, husbandry practices, facilities and schedules has also helped improve our program. Constant oversight and communication is necessary to manage the colony especially when introducing new dogs or merging groups for exercise. We plan to use a computer system to help manage behavioral data. After implementing this program we have increased our success of establishing stable groups, and improved the welfare of our dog colony. Our current colony consists of 59 male beagles in 12 stable groups.

P073 Effects of Individually Ventilated Cages Compared with Conventional Cages upon Body Weighth, Open Field Behavior and Acoustic Startle Response in Wistar Rats

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Individually ventilated cages (IVC) have clear advantages regarding contamination from the environment. Each cage has a tight plastic lid with a hepa filter and the air is usually changed 50 to 75 times an hour. On the other hand, the animals are isolated wit reduced ability to smell and communicate with each other. Many experimental animal models are designed to be sensitive to changes in the animals´ behavior as an effect of environmental changes. Consequently the introduction of individually ventilated cages, may represent a confounding factor. This study was undertaken to compare the effect of housing rats in either IVC or conventional cages upon body weight, open field behavior and acoustic startle response. A total of 48 male Wistar rats were used (weighing 200 to 225 g at arrival). On the arrival day, the rats were separated, weighed and individually placed in either IVC cages (n = 24), or in IVC cages with the plastic lid removed (n = 24). All animals were kept in the same room. Five days after arrival, half of the rats in each group were handled 5 consecutive days for 5 minutes. The IVC rats gained less weight after 17 days ($P < 0.032$) and 34 days (0.0016). The IVC group also showed significantly lower open field activity. The acoustic startle response was only significantly affected with acoustic stimulation of 115dB. Handling had almost no effect on the IVC rats, contrary to significant effects in the control group. The results suggest increased anxiety and reduced anhedonic state in the IVC group.

P074 The Effect of Environmental Enrichment on Time Budgets and Behavior of Socially Housed Long-Tailed Macaques (*Macaca fascicularis*) at in a Breeding Center

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Our institution is a *Macaca fascicularis* breeding center established in Mauritius since 1990. In this study, we evaluated the impact of the set of enrichments on the daily time budget of juvenile monkeys. We used two groups of 25 females Cynomolgus of 26 months of age which were followed over 3 months. The control group was housed in an outdoor pen containing only structural elements while the other group was housed in a similar pen but the latter was environmentally enriched according to Noveprim enrichment plan. The experimental pen is equipped with a variety of devices including: structural perches, platforms and ladders to promote locomotion as well space occupation and positive social behaviors. Puzzle feeders filled with mix of grains are used in the morning, while fruit puzzle feeders are supplied in the afternoon to increase foraging time. Free toys and ropes are available throughout the day for the cynos to play with, encourage object manipulation and provide sensitive stimulation. Each group was observed using 30 second interval scan samplings. The behaviors detected were categorised into subsets according to a previously defined ethogram. This allowed us to establish for both groups a time budget representative of the day. This poster describes the enrichment program implemented at our institution and its impact on the daily time budget of socially housed Cynomolgus.

P075 Effects of CO_2 Euthanasia on Conscious Telemetered Guinea Pig Electrocardiogram (ECG) and Distress

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Carbon dioxide (CO_2) is one of the most commonly used euthanasia agent for rodents. Despite this popularity, CO_2 can cause pain and distress in these animals. We therefore evaluated if a rising CO_2 concentration could be used as a refinement of the euthanasia procedure in guinea pigs, by studying physiologic parameters with telemetry. Telemetered male Dunkin Hartley guinea pigs were divided in two groups (n = 6 to 7). After registering a basal period, animals were introduced in the CO_2 chamber (EHRET CO_2 Box) and were exposed to two different protocols: 100% CO_2 (flow rate of 50 L/min) compared with 80% CO_2 (20 L/min) + 20% O_2 (5l/min). Signs of distress and cardiovascular responses were monitored 1 hour prior to the euthanasia, and time to loss of consciousness and death were recorded following CO_2 exposure. The 100% CO_2 group lost consciousness during the first minute (59.8 ± 4.8 sec) whereas 80% CO_2 group lost consciousness later (97.6 ± 4.8 sec). All animals died during the first 10 minutes period in the chamber with 100%CO_2 (379 ± 62 sec) but just 50% of animals died after this period with 80% CO_2 (503 ± 146 sec). No signs of distress (eye scratching, hemorrhage, eye tearing, etc.) were observed in either group. Heart rate increased when animals were introduced in the chamber (30% increase compared with basal), prior to gas entrance, but rapidly decreased after CO_2 exposure (23% reduction after the first minute), with no significant differences. Death appeared earlier in 100% CO_2 group (379 compared with 503 sec). We demonstrate that, in these experimental conditions, 100% CO_2 exposure leads to safe euthanasia, earlier loss of consciousness and less distress signs in guinea pigs.

P076 Effect of Wire Compared with Solid-Bottom Caging with Bedding on Heart Rate, Activity and Food and Water Consumption in Conscious Telemetered Guinea Pigs

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Rodent toxicology and safety studies have historically been performed in wire-bottom cages (WBC). Concerns over animal welfare have however been raised because, under these conditions, animals are unable to behave in a species-specific manner. Moreover, the *Guide for the Care and Use of Laboratory Animals* recommends solid-bottom caging (SBC) with bedding. The aim of this study was to evaluate potential variations in stress-related physiologic parameters (heart rate, activity, body weight and food and water consumption) in guinea pigs placed on WBC compared with SBC. Dunkin Hartley male guinea pigs were surgically implanted with DSIÒ transmitters to obtain lead II ECG signal. Animals were divided into two groups (n = 10), and after basal recording in SBC, animals were changed to new cages and held overnight in either WBC or SBC during which physiologic parameters and ECG were recorded. Data obtained were analyzed from 7 hours prior to cage change until 18h post-cage change. Heart rate of WBC group increased with time, being statistically significant after 16-18h ($P < 0.01$). Activity was increased in WBC group without statistical significance. There was a significant difference ($P < 0.02$) with respect to gain in body weight: SBC group gained 12 ± 3 g while WBC group

lost 5 ± 6 g, although consuming more food than SBC (22 compared with 19 g). Water consumption, although nonsignificant, was reduced in WBC compared with SBC group (120 compared with 95 mL). These results show that guinea pigs housed in WBC have increases in heart rate, alterations in body weight and food and water consumption, signs that could be related to a stress response.

P077 Vocal Repertoire of Common Marmosets: Can Ultrasonic Vocalizations Be Used to Assess Welfare?

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Vocalizations in common marmosets (*Callithrix jacchus*) and their relation to behavior are well described. However, the vocal repertoire of these animals not only exists of audible vocalizations but also contains ultrasonic components. It has been demonstrated earlier that rats produce ultrasonic vocalizations (USV) in response to painful procedures and provide thus additional information about their acute emotional state. No data are available regarding the USV produced by marmosets in relation to their emotional state. To fill in this knowledge gap and assess whether measurement of USV can be used to increase their housing conditions or improve procedures we recorded audible vocalizations and USV during different conditions. In more than 400 recordings, we distinguished 13 different audible vocalizations. Of these vocalizations, only 4 defined call extended in various settings into the ultrasonic range. Marmosets produced USV only as extensions of audible vocalizations, but not every audible vocalizations was accompanied by USV. USV were found in negative situations but again only as extensions of audible vocalisations. Analysis of the data show that USVis part of the normal vocal repertoire of common marmosets and do not provide additional information compared to audible vocalizations to assess their acute emotional state.

P078 Medical Training of Minipigs for Stress Control during Wound Healing Research

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Pigs are often used as animal models for skin wound healing research because their skin and healing process closely resemble those observed in humans. For these studies, Göttingen minipigs are useful because of their small size, un-pigmented skin and gentle behavior. Stress has been proved to interfere wound healing and increase healing time, showing both statistical and clinical differences. Wound healing studies can be affected by stress, making the results less reliable and representative. Operant conditioning through positive reinforcement offers excellent results in animal training for scientific purposes. The aim of this study was to develop and asses the efficacies of a medical training protocol in Göttingen minipigs during a wound healing study. Six adult male minipigs were used; Stress during housing and daily handling was assessed individually in each animal using two predetermined charts of stress signs and intensity. Signs were monitored daily since the beginning of the medical training plan, two weeks before the skin wound creation, until two weeks after the creation. Housing stress was minimized by the use of chewy elements in the pen and by allowing the expression of social behaviors maintaining the animals in group for 1h/day. Handling stress was minimized by positive reinforcement using diluted yogurt when the animals kept calm during procedures. Results show a significant decrease in the expression of stress signs since the second day of medical training. Medical training can significantly reduce the stress suffered by the animals on these studies and contribute in obtaining more reliable results.

P079 Evaluation of Deviations on Experimental Results Attributed to the Use of Tricaine Methanesulfonate as Euthanasia Agent in Zebrafish (*Danio rerio*)

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Directive 2010/63/EU incorporates zebrafish (*Danio rerio*) as an animal used in experimental procedures, establishing a model to study human diseases and providing new approaches and perspectives. Certain protocols involve the sacrifice of zebrafish, so the euthanasia method is essential to minimize suffering duration and severity, ensuring a painless death which will not mask or alter the experimental results. Tricaine methanesulfonate (TMS), a benzocaine derivative, diffuses rapidly through gill membranes. It has a fast effect on the central nervous system (CNS) level. The aim of the study was to evaluate possible interferences on the experimental results under histopathological analysis due to the action of euthanasia dose of TMS in zebrafish (250 mg/L TMS and buffer solution: 250 mg/L TMS + 500 mg/L bicarbonate). Samples were processed for histopathology. Animals euthanized with 250 mg/L TMS presented, under optical microscopy, destruction of primary lamellae, hyperemia, edema, exudate and hemorrhage. They showed degeneration of the neuronal tigroid at a nervous level. Under transmission electron microscopy were observed hyperemia and disruption of the capillary walls in the gills. The CNS root neurons showed vacuolated cytoplasm and nuclear pyknosis. Scanning electron microscopy presented transfer and infiltration of exudate and blood cells at a branchial level. Lesions in euthanized fish were less severe when a buffer solution was employed. Hyperemic processes in main lamellae and diffuse edema in the root zone of the spinal cord.

P080 Histopathological Study on the Effects of Euthanasia Dose of Eugenol in Zebrafish (*Danio rerio*)

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When possible, death should be avoided as a proceeding endpoint and replaced by humane and earlier endpoints. Pain, suffering and/or distress elimination is necessary for safe, effective, predictable and appropriate to each species euthanasia procedures, to avoid stress factors that cause physiologic changes that may also lead to unsatisfactory results. The use of zebrafish (*Danio rerio*) has been increased exponentially in biomedical research. Directive 2010/63/EU includes it in the list of animals in experimental procedures. Eugenol, the main active ingredient of clove oil, acts on the central nervous system (CNS), absorbed through fish gills and skin. Our goal was to identify and evaluate tissue damage in zebrafish caused by exposure to eugenol as an euthanasia agent (250 mg/L of eugenol; 1:10 eugenol: ethanol). Samples were fixed for histopathology study. Under optical microscopy gills presented hyperemia, hemorrhage and inflammatory infiltration. At a CNS level samples showed generalized edema and neuronal degeneration, with loss of Nissl bodies. Gills showed, under transmission electron microscopy, capillary swelling with hyperemia, disorganization of the lining cells and increased mucous cells. At a nervous level, coagulation necrosis of the neurons with marked cytoplasm densification and generalized vacuolization of the membranous system. Scanning electron microscopy showed swelling, disruption of the lamellae and presence of inflammatory cells and hemorrhage.

P081 Mice Housed within Individually Ventilated Cages with Circular Air Motion May Become Preconditioned to Hypoxia

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At our institution, researchers discovered unexpected changes in data during an acute hypoxia treatment after mice were housed within individually ventilated cages (IVCs). Coincidentally, our institution was transitioning mice from microisolation and wire-topped shoebox cages (WTCs) into IVCs. The IVC system functioned with air entering through the air inlet located in the back of the cage. Air passed across the bottom, sides, and front of the cage and exited at the top. A study was conducted to compare housing of mice within IVCs to WTCs, which are exposed to ambient air. Three-week old male, C57BL/6 mice were housed 2, 3, or 4 animals per cage for 5 to 9 weeks prior to testing. Intra-cage oxygen within IVCs was significantly decreased (1.69 %, 1.99%, 2.54% respectively) as compared to WTCs during one week of housing. Mice raised within IVCs had elevated red blood cell (RBC) mass, hematocrit, and hemoglobin concentrations and higher platelet counts. A physiologic response to reduced oxygen is increased RBC mass and hemoglobin concentration. IVC housed mice drank significantly less water during the first 24 hours following relocation to WTCs. They had a significantly greater preference for saccharin solution and drank more total fluid during a 24 hour test. IVC housed mice recovered more rapidly than WTC mice from lipopolysaccharide-induced loss of locomotor activity. When considering housing laboratory mice within IVCs, one must take into account the air ventilation system of the cage and realize that a slightly hypoxic condition may result and impact hematopoietic and behavioral research.

P082 Histologic Changes after Retrobulbar Injection

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The use of retrobulbar injection is still under debate concerning animal welfare. To investigate histologic consequences of retrobulbar injections we performed histologic sections of the whole eye two days and 14 days after retrobulbar injection of marker substances for Glomerular Filtration Rate (GFR) measurement and saline respectively (130 µL/animal). The retrobulbar injection did not cause any visible local ophthalmologic complications or create any behavior changes. Thus, this procedure proved to be suitable concerning clinical standards. The retrobulbar injection led to small areas of necrosis due to tissue damage by the canula in the harderian gland ore in one case in the eye muscle (histologic results after 2 days). The infiltration of mononuclear cells showed that the region of necrosis was going to be demarcated. A scar like lesion and a – may be transient- loss of function of the organs involved (that is, harderian gland, eye muscle) can be anticipated. However, the vitreous body, retina, cornea and eye nerve were not involved in any case. Transcutaneous GFR measuring resulted in reliable data by caudal vein-injections as well as by retrobulbar injections of the marker substance. In our case the retrobulbar injection would be preferable to the caudal vein-injection for application purposes. Even for animal welfare purposes it may be recommended since the animal is under quick inhalational anesthesia and is not stressed by warming up procedures, fixation or repeated injections (often necessary with caudal injections). The histologic examination of eyes 14 days after injection to evaluate permanent or irreversible changes is not completed yet.

P083 Assessment of Intraplacental Euthanasic Overdose in Third Trimester of Gestation Mice Fetus as a Humane Euthanasia Method

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Directive 2010/63/EU on the protection of animals used for scientific purposes includes fetal forms of mammals as from the last third of their normal development, so effectives and human methods of euthanasia shall be applied. AVMA Panel on Euthanasia report provides limited recommendations for the euthanasia of prenatal animals, and states that when ovarian hysterectomies are performed, euthanasia of fetus should be accomplished as soon as possible after removal from the mother. Rodent fetuses are resistant to hypoxia, exhibiting heart beat movements up to 40 minutes after mother death. Decapitation should be a valid method, but anatomic structures are compromised. Doe to fetus pain perception should be considered, other euthanasic method efficacy has been evaluated. Our aim is to assess the effect of intraplacental Pentobarbital overdose as a way to allow obtaining anatomic complete fetus avoiding death by hypoxia.6 gravid CD1, C57BL6 and 129sv mice (E16) were sacrificed by cervical dislocation. Uterus was exposed by abdominal longitudinal incision. Pentobarbital 400 mg/kg was microinjected in at least two-per-mouse fetal placenta blood exchange region. Noninjected fetuses were considered as their own controls. Permanent cessation of heart contraction was confirmed by ultrasound techniques. Results showheartbeat depression and arrhythmias after 5 min post-injection. Cardiac arrest arrived 12,4 minutes average post-placenta injection. Control fetus remained efficient heart beating up to 40 minutes. Regarding the results, we pose Pentobarbital intraplacental overdose injection seems to be an effective euthanasic method for fetus, causing neither hypoxia nor anatomic compromise.

P084 Evaluation of Mouse Behavior Regarding 2 Different Types of Bedding Using a Motor-Free Ventilated Caging Block System: Preliminary Results

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Bedding material is an important factor influencing the health and wellbeing of animals as well as the quality of experimental results. The aim of the study was to evaluate the cage behavior of mice in cotton packed wood particle or corncob bedding. The evaluation test was performed using a system of two motor free ventilated cages connected with a plastic tunnel. A total number of ten male and ten female C57Bl6/J mice at the age of ten weeks old were used. All groups were videotaped during the light cycle (07:00 to 19:00) for five consecutive days. Food and water consumption of animals as well as the initial and final weight of the cages with the bedding material were measured. Based on the preliminary results from the video analysis, male mice cross the tunnel from the corncob bedding to cotton paced wood particle quicker than the females did. During the second observation day, both male and females had formed a nest using the cotton pack and after the third day, animals began to mix bedding and move freely between both cages of the block. No difference in food consumption was observed while there was a statistically significant difference in water consumption. It is concluded that male mice have a greater tendency to explore their environment, all mice initially prefer cotton pack bedding but eventually, the animals become active in all cage areas and mix the bedding material.

P085 Humane Endpoints in Aging Rodents

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Research related to the aging process is increasing rapidly and with that the demand for aging rodents. Aging mice and rats have a higher risk of developing symptoms that may cause discomfort to these ani-

mals. To stay alert in the prevention of unnecessary discomfort, more than we currently do, it is suggested to introduce humane endpoints specific for the aging mouse and aging rat in addition to the generally applied humane endpoints as listed in for example, the OECD guidelines. On the other hand one should beware of taking action too fast - you do not want to euthanize animals that have a phenotype that is normal for aging rodents. For instance a rough hair coat, light forms of cataract, and animals that are somewhat obese or somewhat skinny but function and behave fine. The estimated average time at which mice could be considered being 'aged' is 1 year (average life span divided by 2); 1.5 years for rats. We propose the following specific endpoints: evidence of the presence of tumors (visible or palpable), evidence of fighting with visual wounds, significantly disturbed mobility, animals (gradually) do not keep a normal body weight, abnormalities (like severe cataract) or loss of one or both eyes, animals remaining (single) from an age-matched group because the others have been used/sold/died and regrouping is not an option. Using these humane endpoints when working with aging rodents will help to maintain a high standard of animal welfare in aging research.

P086 A Welfare Assessment Framework for Laboratory-Housed Beagles

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The link between good welfare and good scientific output is often made although lacks supporting data. Decisions relating to housing, husbandry and scientific procedures are often based on anecdotal rather than empirical evidence. This ongoing collaborative project between academia and industry examines the link between welfare and quality of scientific output (repeatability and variability) in laboratory-housed beagles (>2,800 were used in scientific procedures in the UK in 2011, primarily for toxicology). A crucial first step was developing an integrative framework for welfare assessment to investigate the effects of planned Refinements to areas of key interest, namely housing, desensitisation and assessing the impact of scientific procedures. This study utilized measures of affect (that is, cognitive bias), behavior (that is, behavioral states and stereotypies), cardiovascular data from implanted telemetry (that is, heart rate and blood pressure) and mechanical pressure threshold testing to identify measures which are indicative of welfare changes and quantify the impacts on quality of scientific output. To investigate the influence of housing and husbandry systems (for example, staff contact, history of licensed procedures) on welfare, groups of dogs in different housing areas were compared. Observations in the home pen identified suitable behaviors which were then measured in response to behavioral 'challenges' to identify those most sensitive to changing welfare. Results identified differences in welfare and provided easy-to-identify behaviors associated with changes in affect, patterns of behavior and cardiac parameters, providing an empirical basis for further Refinement research and a means for technical staff to monitor welfare and impact on quality of scientific output.

P087 Enrichment Challenges and Solutions in Toxicology Studies

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Throughout the UK research industry, animal enrichment is used in various forms and for nearly all types of studies. In the rest of the world, however, the usage varies. In some establishments, the cages are enriched with all manner of items and may even be busy to the point of impaired visibility. In others, such as toxicology laboratories, the cages are still barren by UK standards. For toxicology studies, there are valid concerns on how enrichment may affect study outcomes. While it is widely recognized that enrichment of home cages is in most cases necessary, restrictions need to be considered such as exactly what is being introduced into the cage and how these substances may interact with study compounds. This poster aims to review the challenges faced by toxicology studies and how the industry has advanced the availability of GLP enrichment items. It also reviews some of the incidental and empirical evidence for various types of enrichments that have been published

P088 Single Housing of Male Mice Affects Serotonergic Signaling Integrity as Measurable Through 8-OH-DPAT Induced Hypothermia

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A much debated topic in housing laboratory mice is the need for cage mates. In particular, male mice are often housed singly because of their aggression toward other males, meaning that social isolation of laboratory mice is an issue that needs to be explored further. The biggest hurdle that must be negotiated is how to identify and quantify the negative impact brought on by single housing. In the present study, male mice that had been single housed for a period of 32 days in conventional open cages were found to respond with a more marked hypothermia to a challenge with a selective serotonergic agonist (2 mg/kg bodyweight 8-OH-DPAT) than their group housed counterparts ($F_{4,31} = 3.50$, $P < 0.05$). Isolation-induced enhanced activity of the target receptor (5-$HT_{1A)}$, leading to an amplified hypothermic effect, is highly associated with depressive states in mice and humans alike. We therefore suggest that the challenge can be used to demonstrate the negative emotional state brought on by long term social isolation in laboratory mice. The study emphasizes the importance of cage mates; that even male mice not only choose social contact when given the option, as has previously been shown, but will also, when it is deprived, be negatively affected by its absence. Whereas the method requires additional study it potentially allows not only for an unbiased, biochemical evaluation of sub-acute stressors, but allows for determining whether these detrimental effects can be counteracted, for example through enrichment of the animals' environments.

P089 Gel delivery System for Oral Voluntary Administration of Buprenorphine in Mice

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Oral administration of peri-operative analgesia to laboratory mice is beneficial compared to administration by injection. The mice become less stressed and receive high and long lasting serum concentrations of the drug, when allowed to voluntarily ingest the drug in a palatable feed item. We have demonstrated sticky nut-and chocolate paste to be well-liked by mice and ingested in almost all cases. However, a disadvantage with nut- and chocolate paste is its high content of fat and sugar, which may have undesirable effects in some experimental models. An alternative to nut- and chocolate paste could be a gel delivery system, using a gel consisting of approximately 98 % water to serve as a supplementary source of fluid post-operatively and as a vehicle for analgesic drugs. In the present study, we investigated the willingness of mice to ingest water gel, regarding the duration from introduction of the gel to first ingestion, as well as the amount ingested overnight. Furthermore, various concentrations of buprenorphine were mixed in the gel, and the subsequent serum concentration of buprenorphine was investigated. It was found that the water gel was ingested by the mice, but with less willingness compared to ingestion of nut- and chocolate paste. The serum concentrations of buprenorphine were similar to those after subcutaneous injection, but the variation was considerably higher. In conclusion, water gel may serve as a relevant vehicle for oral administration of buprenorphine, but further studies are needed to improve the willingness of the mice to ingest the gel.

P090 Is It Still Justified to Use CO_2 for Euthanizing Laboratory Ani-

mals? A Comparative Study to Inhalant Anaesthetics

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Carbon dioxide ($CO_{2)}$ can be still used for euthanizing laboratory rodents but the method is strongly criticized. Inhalant anaesthetics are recommended as an alternative, but their application is not sufficiently validated. Here, we investigate distress induced by 100% CO_2 with different filling rates (20% (CO_2 20), 60% (CO_2 60), CO_2 100% (CO_2 100) of chamber volume/min) or isoflurane and sevoflurane in different concentrations (Iso2%, Iso5%, Sevo4.8%, Sevo8%) in NMRI and C57Bl/6 mice. We evaluated all gases for their effectiveness and reliability to induce general anesthesia within 300s. We observed the behavior during the induction of narcosis and measured plasma concentrations of adrenaline and noradrenaline immediately after surgical tolerance was reached. Only CO_2 60, CO_2 100 and Iso5% induced general anesthesia in all animals of both strains within the given time. Surgical tolerance was reached faster by CO_2 60 or CO_2 100 compared to Iso5%. All NMRI mice but not all C57Bl/6 mice exposed to Sevo8% were anaesthetized within 300s. Behavioral analysis revealed no distinct signs for distress during the induction of narcosis. Adrenaline and noradrenaline concentrations were significantly increased in CO_2 treated animals compared to animals exposed to isoflurane or sevoflurane. CO_2 60, CO_2 100 and Iso5% effectively and reliably induced general anesthesia, whereas sevoflurane was not as effective. However, the raise in adrenaline and noradrenaline plasma concentrations after CO_2 exposure points to distress in the animals, so CO_2 alone is not the first choice for euthanizing laboratory mice. Isoflurane should be used with the maximum concentration to provide a save stage of surgical tolerance. Supported by the Bundesinstitut für Risikobewertung-ZEBET (FK 3-1328-429).

P091 Management of Behavioral Issues in the Laboratory Mouse: Barbering and Aggression

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Barbering and aggression in mice are common behavioral problems in the laboratory setting. Barbering implies the removal of whiskers and hair from one mouse to others or to itself, and it seems to be a caused by multiple factors, including genetic predisposition and environmental stress. Aggression is of particular prevalence in male mice, and different measures have been proposed to minimize this problem. Although in a low prevalence, both behavioral issues described are found in our center; in this study we present the results of our approach to minimizing barbering and aggression in 2011 to 2012. The two strategies we have used so far are: 1) identifying and removing, when possible, the animal causing the behavior, 2) adding extra environmental enrichment (running wheels) to try to keep the animals active and away from the undesired behaviors. Removing the less barbered individual from the group was the most effective strategy to reduce barbering: 50% of the groups from which the suspected barber was removed showed a clear improvement. Addition of extra environmental enrichment after the onset of the behavior had no effect. Removing the aggressive individual from the group resolved aggression issues in most cases (88%), although in some cases fighting continued between the remaining animals (11%). In conclusion, up until now the best strategy for managing both barbering and aggression is the removal of the animal causing the behavior. Proposals for future improvement in the management of these behavioral problems are also discussed.

P092 Pup Mortality in Laboratory Mice: An Ethological Approach

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Perinatal mortality is a relatively common problem when breeding mice, reported mortality rates vary from nearly 0 to 50% in experimental studies. However, knowledge of what causes perinatal mortality is scarce. Pup mortality constitutes a welfare problem and leads to an increase in the number of breeding animals needed to supply experimental animals, contradicting the goal of reducing the number of animals used for experimental purposes. Dead pups are generally eaten and it is commonly assumed that mothers kill their pups. The aim of this study was to increase the understanding of litter mortality and investigate if females actively kill their pups. We used video recordings of 10 females (C57BL/6 and knockouts $Hfe^{-/-}$ and $\beta 2m^{-/-}$) that lost their litters to observe interactions between mother and pups from parturition until the pups died. A flowchart was used to systematically focus on critical events. Females interacted with both moving and still pups, but were never observed manipulating a moving pup that stopped moving after the interaction. Nor were any wounds visually detected during the video observations. Hence, even with detailed observations, we found no evidence that females kill their pups. These findings highlight the importance of being cautious when concluding how laboratory mouse pups die, and stress the need for more systematic investigations. By assuming that pups are killed by the mother, based on findings of pups half-eaten or not found at all, the true causes of pup loss are probably overlooked, and a welfare problem in laboratory mice is left unresolved.

P093 Telemetry Recording in Group-Housing Large Animals in Compliance with the New Directive 2010/63/EU

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We have recently designed new facilities for housing animals dedicated to experimental purposes (efficacy and safety pharmacology studies). Even if the requirements for the care and accommodations of the animals should be implemented only before January 2017, we decided to directly fully comply with the new Directive 2010/63/EU to ensure animal welfare and to implement the 3Rs rule. However, as a CRO, our challenge was also to strike a balance with good science and competitiveness. Nonhuman primate (NHP) and dog facilities were designed for group-housing in large modules to promote social interaction and natural hierarchy. Various textures with platforms, ladders and toys are used as enrichment. Our main activities in these facilities are focused on cardiovascular evaluation animals using telemetry, the gold standard technique to obtain physiologic data in freely-moving conscious animals. In order to avoid restraining and maintain animals in their large home cage, key factor to decrease stress and increase animal welfare, we benefited from recent technical improvement of telemetry hardware, including multifrequency and long range digital signal, allowing telemetry acquisition in group-housed animals in large modules. In addition, we developed in NHP, operant conditioning procedures with positive reinforcement to obtain active participation of the animals and further decrease stress during experiments. Finally, high definition video monitoring is used to evaluate drug effects on animal behavior, thus avoiding artefacts linked to the presence of the experimenter in the experimental room. In conclusion, high quality telemetry recording is possible in large animals while complying with the new directive.

P094 Best Practices Development for the Use of Nonhuman Primates in Research: The Association of Primate Veterinarians as an Educational Resource for Enhancing Primate Welfare

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The Association of Primate Veterinarians (APV) recognizes the continuing need for judicious use of nonhuman primate models in scientific research for the foreseeable future. Because of their advanced cognitive capacity, APV accepts that nonhuman primates can be difficult to manage well in captivity, that research use should be highly scrutinized and limited to the most essential studies, and that accepted standards and conditions for management of these species in captivity must be constantly reviewed and refined to enhance animal welfare. There are no globally accepted standards for nonhuman primate care and use, which may result in deficiencies in animal wellbeing. To achieve international harmonization of high standards of care, APV is committed to promoting excellence in nonhuman primate knowledge, care, and responsible research use. A key role of the organization is to develop, educate, and disseminate best practices for refinements in nonhuman primate care and management. APV has developed and published a number of guidelines documents to assist veterinarians, researchers, and animal care committees to enhance animal wellbeing, including those for social housing, care of cranial implants, use of food and water restriction as research motivators, and . The organization also promotes a number of other educational tools, symposia, fellowships, and international veterinary exchanges to further the knowledge of those working with these species. This poster will describe APV's processes for developing best practices to refine the care and use of nonhuman primates.

P095 Development and Continuous Improvement of a Program of Environmental Enrichment for a BSL3 Animal Unit

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Environmental Enrichment refers to modifications of the environment of captive animals that improve their welfare. In our center, a program of environmental enrichment for rodents (mice and rats) has been in place for the last 6 years. In this communication, we describe studies carried out to set the program and also to continuously review and improve it. The specific characteristics of our BSL3 facilities, such as work with highly immunodeficient mice and housing infected animals in isolators, make the use of environmental enrichment particularly challenging. The first set of environmental enrichments we tried were nesting materials and cardboard shelters. Initially, nesting material was considered the most appropriate material for mice and we showed that it did not interfere with physical and physiologic variables. However, they had to be discarded after one year of use because they interfered with the automatic system for bedding disposal. Rats did not build nests with the nesting material, thus it was not considered a good choice for this species. Both mice and rats destroyed cardboard shelters too quickly to be considered a good option. Therefore we decided to evaluate different types of rigid autoclavable shelters for both mice and rats, that were also compatible with the functioning of the facility. After trying many options, the devices the animals preferred were tinted igloos for mice and hollow balls for rats. We have been using these autoclavable materials for several years now. On-going work on specific solutions for particular issues, such as cannulated animals, is also described.

P096 Characterization of Gene Expression in Naturally Occurring Feline Degenerative Joint Disease Associated Pain

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Degenerative joint disease (DJD) associated-pain is a clinically relevant and common condition affecting domesticated cats and other species including humans. Identification of the neurobiological signature of pain is well developed in pain models in rodents however this information is lacking from animals or humans with naturally occurring painful conditions. In this study, identification of housekeeping genes (HKG) for neuronal tissue and expression levels of genes considered associated with chronic pain in rodent models were explored in cats with naturally occurring osteoarthritic pain. Eighteen adult cats were evaluated – 9 normal, and 9 with hind limb radiographic DJD. Expression of pain signaling genes (including *Nav1.7, Nav1.9, NGF, NK1, TNFα, TrkA, ASIC3, COX2, Nav1.8, CX3CL1, ATF3*) in lumbar spinal cord dorsal horn, lumbar dorsal root ganglia and joint capsule tissues from normal and DJD-animals were studied using quantitative RT-PCR (qPCR). HKG identified as the most stable across all tissue samples were many of the ribosomal protein genes, such as *RPL30* and *RPS19*. qPCR results showed downregulation of *COX-2, NGF* and upregulation of *TNFα* genes in spinal cord and downregulation of *TNFα* in dorsal root ganglia collected from DJD-animals. No genes were differentially expressed in the joint capsule samples due to high levels of variability in the healthy samples. Several genes expected to be upregulated, based on results in rodent models, were downregulated. Further work is needed to understand the neurobiology of pain in naturally occurring disease and what rodent models are predictive of these changes in more heterogeneous populations such as domestic cats.

P097 Primate Adoption: An Essential Alternative to Hand-Rearing

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Rejection, poor nutrition and maternal death are among a range of reasons why an infant primate might require special attention if it is to survive during this period of particular vulnerability. A common response of managers and care-givers is to intensively hand-rear such infants until a time when they can feed themselves. However, hand-rearing itself can produce many problems including imprinting on humans and poor or inappropriate development of species-typical social and sexual behavior. This is a particular problem where hand-reared animals are to be returned to the company of conspecifics. Bioculture is a breeder of high quality, naturally SPF long-tailed macaque (*Macaca fascicularis*) research models with 7000 breeding animals and as many as 4000 births per year. Bioculture has a policy to avoid hand-rearing as it is not considered to be in the welfare interests of the animal, instead it employs a very successful program of placing infants in need of assistance with adoptive mothers in existing social groups. Less than 1% of all live births at Bioculture require consideration for adoption. Of all of those given for adoption 87% are fully and successfully adopted. This presentation will describe the adoption process at Bioculture and examine the key decisions and factors that determine its high level of success.

P098 Replacement of 40% of the Cages (from Filter Cover to Individually Ventilated Disposable Cages) Turns into a 20% Increase of Capacity with No Extra Expenses

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The need to increase space to allocate animals without rebuilding the 800 m^2 SPF mice animal facility and without increasing the operational cost (running annual expenses), has led our Institution to decide replacing the 40% of the filter cover cages by ventilated disposable cages, in 2011. With disposable cages (with bedding and plastic water bottle), some activities have been reduced: such a washing, sterilization and husbandry. The space to allocate clean cages in storage or the level of

dust, ammonia and CO_2 in the room has also been reduced. Even though the amount of waste has increased, the plastic cages are recycled with no extra cost. The animal welfare has improved (animals build their nest under the water bottle), the risk of ergonomic lesions of the cleaning personnel and animal care has decreased and technicians spend 15% less time in husbandry activities. In the Biosafety II gene therapy room the sterilization activity has been reduced by 50%. During 2012, the personnel has been reduced by almost 10% (half salary from cleaning personnel and half salary from animal care technician). The animal facility capacity increased a 20% without increasing the annual expenses.

P099 Design of an Animal Facility Integrated in a Molecular Imaging Unit

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In vivo molecular imaging techniques are perfectly suited to approach biologic, physiologic and/or medical questions via longitudinal studies. Due to their noninvasive character (Refinement) the same animal can be scanned repeatedly, improving thus the statistical significance of the results while the number of animals is minimized (Reduction). However, small animal scanners are usually sited out of the animal facility; this fact leads to a controversial situation, because animals have to enter and leave the housing rooms to undergo the experimental procedures. At our institution, a fully equipped molecular imaging facility capable to run PET, SPECT, CT and MRI studies in small rodents (rats and mice) is available. As the center is not attached to a big animal facility, the design and implementation of an animal housing unit was envisaged during site planning. he final solution required compromises to guarantee animal welfare and operational viability, while minimizing the risks of cross-contamination and potential infections. Despite space constrictions, the design of the animal house in two different areas, appropriate control of the environment through air filtration and temperature and humidity control, the implementation of a pressure cascade and appropriate Standard Operating Procedures to ensure a proper workflow have permitted to run longitudinal experiments in different animal models (including inmunodepressed mice) without adverse symptoms.

P100 Effects of Individually Ventilated Housing in Telemetry-Implanted Guinea Pigs

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Individually ventilated cages (IVC) have some well-known advantages with respect to the nonventilated open top cages (OTC), although some concerns have recently arisen about their potential harm in rats and mice. The aim of the present study was to evaluate the potential alterations in some stress-related physiologic parameters such as heart rate (HR), locomotor activity (LA), body weight (BW) and food consumption, in telemetry-implanted guinea pigs housed in IVC. Dunkin-Hartley male guinea pigs were surgically implanted with telemetric transmitters (DSI) to register the heart rate (HR) and the locomotor activity (LA). Animals were moved from an OTC to a new, freshly prepared OTC (n = 10), or to an IVC (n = 10). Telemetric parameters were recorded continuously during 5 days from that cage change. BW and food consumption were recorded every other day. When comparing HR values obtained in OTC and in IVC, no relevant changes were observed at any moment of the study (mean ± SD: 207 ± 9 bpm and 213 ± 10 bpm, respectively). The main differences were observed in LA, with a greater activity in IVC-group during the first 4 h after the cage change ($P = 0.004$), and lesser during the rest of the day and the following night ($P \leq 0.001$). Animals housed in OTC consumed more food than IVC group ($P < 0.01$). In conclusion, IVC housing induced a transient less active status in guinea pigs, without an increase in HR, neither BW loss, which suggests that it does not represent a stressful experience for the animals.

P101 Comparision of Conventional Cages Compared with GR1800 Double Deckers and Welfare Benefits for Rats and Gerbils

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Liverpool University has recently opened a state of the art SPF facility to replace its old conventional unit. We have moved from open conventional caging to Individual Ventilated Cages (IVCs) for all our rodent species. Preliminary observations reveal improved welfare benefits for our Jirds (Gerbils) in reduced incidence of stress related nasal dermatitis associated with *Staphylococcus aureus* and implicated in *Staphylococcus xylosus* etiology. We have also observed a more predictable and reliable littering down by our time mated Wistar rats that we believe is partly due to the high visibility offered by the use of the GR1800. This has scientific benefits for one of our research groups as the female can be observed without disturbance and the uterus taken at early signs of parturition. The benefits of this translate into the prolonged maintenance of a contracting uterus for up to 24 hours resulting in increased data output and lower numbers of animals being used in line with the ethos of the 3Rs. This presentation provides an overview of our findings as well as discussing the environmental changes in provisions that need to be considered when switching from conventional caging to IVCs.

P102 Optimising Facility Design and Operations (More Science for Lower Operating Cost)

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Facility design should provide value for money in facility construction cost, enable lower running costs, reduced staffing numbers and allow for more time to be spent on animal welfare and science and less on operations. However, too often, facility design defines many spaces by 'rule of thumb' or from the client users, designers, or design companies own experience and knowledge. The design may often be undertaken with limited information on the likely facility procedures. Therefore it is difficult to validate aspects of design and compare the various design and cost options which, to different extents, affect operating procedures. This presentation will show the relationship between operating procedures, layout design and equipment specification. Then examine how early stage documentation of procedures and the validation of design elements and equipment by using calculation and recorded data, can optimise the design at the briefing stage. This clarifies the client's explicit requirements before the main design is procured. We will illustrate how using "shadow studies" provides the design team with a good understanding of the operational needs of each specific facility and allows us to offer operational enhancements based on actual protocols and procedures. We aim to show that, the using knowledge-based design solutions with quantitive data are essential in ensuring the design is proved to have captured the brief requirements. We will also illustrate how the concepts of "lean management" can contribute to lower operating costs and improved efficiencies.

P103 Key Performance Indicators in the Animal Facility. A Useful Management System for Cost Containment, as Well as a Means of Animal's Welfare Evaluation

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Since 2004, in our Animal Facility we have been working with different indicators included in an annual balanced scorecard. We have been using 20 parameters to monitor the performance of our activity. In this communication we will present the results obtained from the Key Performance Indicators (KPI) used in our animal facility. (1) Customer perspective: researcher's annual satisfaction survey. During the period 2007-2011, the average score obtained has ranged between 4 and 4.41 out of 5. (2) Financial perspective: unit cost of cages (euros/cage/day). Even though the percentage of cages has increased almost a 200% between 2004 and 2012, the unit cost has decreased a 5.2%. (3) Business process perspective: ethical review process and husbandry and veterinary care activity. (3. a) Percentage of unfavourable animal protocols by the competent authority. Once evaluated by the IACUC, 40 animal procedures are sent for assessment every year. Since 2004, the Competent Authority has fully rejected 1% of the animal protocols, and 10% have been approved after providing the requested additional information. (3. b) The husbandry and veterinary care activity is measured in terms of dead or moribund mice found. During the period 2011-2012, the average score has been 30 dead mice found per year in 100 cages. (4) Learning and growth perspective: training investment. This data shows the invested budget in training and education. Since 2006, the expenditure on training per employee has tripled. This management system has allowed us to promote education and training, continuous process improvement, maintaining high rate of employee researcher satisfaction and promoting animal welfare, without increasing costs.

P104 Upgrading the Health Status in a SPF Facility without Any Renovation and Downtime

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Upgrading mouse facilities is nowadays necessary to improve the health status of mice, and thereby generate reliable and reproducible mouse models characterization. Improving the microbiological status of animals also facilitates the exchange of mice between mouse facilities. Most of the time, upgrading facilities involves hard renovation, downtime and therefore important operational costs. As a mouse mutant production and banking center for the scientific community, we initiated a program to improve the health status of our breeding facility based on rederivation through embryo transfer. These rederivations were conducted in our SPF facility endemically infected by some opportunistic agents. We set out here a straightforward solution to improve the health status of mice without any hard renovation and downtime, based on custom designed, soft walled, positive pressure class 100/ISO 5 clean room with appropriate procedures.

P105 An Easy Ergonomic Way to Lift and Move Anaesthetized Pigs and Sows

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Pigs are used extensively in experimental surgery and when preparing the animal for surgery it is important to be aware of good ergonomics in order to prevent work related injuries in the staff, but also to move the pigs around in a gentle way. At our Department we have established a procedure, where we are able to handle anaesthetized pigs from 20-350 kg. We can transport the animals to and from the operating theatre without dragging, pulling or pushing the animals eliminating the risk of damaging the animal and without any manual lifting by the staff. The pig is anaesthetized in its home pen to minimize stress. When the pig is in lateral recumbency it is gently rolled onto a sling for further transport by means of an overhead lift mounted in the ceiling or by a mobile lift. In this way one person, or two persons for larger pigs, can easily lift and move pigs around gently and without a strenuous work effort.

P106 Suitability of a Fish Standalone System to House *Xenopus tropicalis*

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Xenopus tropicalis frogs are social animals and can be kept in large communal groups, as they are neither territorial nor try to establish hierarchies, unlike other species. Due to changes in our research direction we had to increase the fish holding capacity within our Institute while continuing to accommodate a smaller colony of frogs in the existing aquatics foot print. To allow us to utilize the space most efficiently we carried out trials on a system that used smaller tanks designed to house zebra danio fish. Our investigations looked at any potential detrimental impact to the health, behavior and scientific data of the frogs we house. Through using our knowledge of working with this species, we have demonstrated that we were able to provide a suitable alternative environment using a fish aquatic system. We ran a trial using a standalone fish system with smaller tanks, and smaller group sizes and housing them in tanks of differing volumes of water. During the trial we were able to provide the frogs with their normal feeding and enrichment regimes. Here we present the findings of our trials and how aquatics systems used in a suitable way can be adaptive to a variety of situations.

P107 An Introduction to VIDO-InterVac

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The Vaccine and Infectious Disease Organization - International Vaccine Center (VIDO-InterVac) is a nonprofit research organization situated in Saskatoon, Canada. Our research is focused on infectious diseases and vaccine development for both human and animal health. This includes the development of novel animal models for human and animal diseases including surgical, nonsurgical and infectious disease models. For example novel disease models have been developed here for pertussis, respiratory syncitial virus, and intestinal diseases to name but a few. We have recently opened the International Vaccine Center, perhaps the newest and most advanced BSL3 laboratory in the world today. Designed specifically for the development of vaccines against CL3 infectious diseases, this facility has 13,500 m^2 of floor space with 1500 m^2 of CL2 and CL3 laboratory space and 1300 m^2 of CL3-Ag animal containment. It can support single or multiple pathogens involving multiple investigators and animal studies can range from single room up to sixteen room trials and involve multiple species. VIDO-InterVac is an international facility open to researchers from academia, industry and governments from around the world and is designed to house a variety of animal species including nonhuman primates, large animals, poultry and rodents. Every effort has been made to ensure that it is "animal friendly" to address animal welfare concerns. This presentation will highlight some of our research as well as some of the novel design features that facilitate safe movement of animals while supplying them with high levels of comfort and security.

P108 Ten Years of Experience with Building-Integrated Ventilated Caging Systems: What We Have Learned

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In 2003 the University of Michigan opened the first of its two large vivaria that were engineered to have rodent racks ventilated directly by

the building's air supply and exhaust systems. The overall outcome of this effort has been extremely successful: cages are ventilated steadily by filtered air, the rooms are quieter and more pleasant work places, and rodent breeding has been successful in colonies in which previous rack-mounted blowers negatively affected the birth/weaning rates. Use of the building ventilation system has benefitted the animal care staff ergonomically by eliminating the need to move blowers off/on the racks on change days, and has eliminated the labor associated with cleaning the blower pre-filters. It has also been subjectively noted that cages integrated with the building ventilation system need fewer "as-needed" changes prior to the regularly scheduled change days. Another positive outcome of the integrated system has been a closer working relationship between building maintenance staff and the animal facility managers. Our systems were designed with separate thermostats for room air and intra-cage air, in an effort to control cage temperature independently from room temperature (that is, warmer cages for mice with cooler rooms for staff). Unfortunately this goal was not achieved, as the temperature of the air provided to the cages was readily influenced by the temperature in the room. A review of our study on the effects of differing thermostatic set-points, including measurement of air temperatures at multiple locations within the rack supply plena, will be presented.

P109 Reptiles as Experimental Animals: Challenge for Researchers

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Reptiles are a group of about 10,000 species living today, which play a key role in the environment. They render valuable ecosystem services such as the control of pest species, dispersal of seeds, pollination and are food for other animals or humans. They are also becoming more and more popular as pets. Unfortunately, their very existence is threatened by illegal catches, overexploitation, loss of habitat, pollution and climate changes. Many reptiles are on the IUCN Red List of Threatened Species or are covered by the provisions of the CITES. The knowledge about the biology of reptiles, especially mechanisms to adapt to their environment, the diversity of pathogens and diseases, can not only help restore the population of endangered species but also give veterinarians a tool to improve the health status of pets and wildlife as well as protect people from zoonoses. Over the last years, the scientists' interest in the influence of environmental and pathologic factors on reptile reproduction and development has increased considerably. However, due to the nature of this group of animals, researchers are faced with many constraints limiting the studies. This poster presents the critical points of the study plan related to, among other things, the international and local law and reptilian physiology. Some examples of design solutions for the construction, enrichment and adjustment of an experimental terrarium to different species are also presented.

P110 Temperature Control during Rodents' Surgery: A New Approach

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Most procedures in research have to be performed using anesthesia. However, anesthesia reduces the metabolism of animals and consequently body temperature, leading to death by hypothermia. Therefore, monitoring and control of body temperature is mandatory during anesthesia. In addition, during surgical procedures it is important to have an easy to clean surface to reduce contamination. This is a problem with current available solutions to control animals' temperature. Furthermore, we have recently showed burns with homoeothermic blanket systems in mice. The purpose of this study was to evaluate new equipment, which has a temperature unit control integrated in the operating table. To compare this equipment with current homoeothermic systems, eight female mice with four months of age were divided in two groups: 4 mice for a current market solution and 4 mice for the new equipment. The mice were anesthetized with isoflurane during 30 minutes using a coaxial mask and put above the heating areas of the devices. Body temperature was measured continuously during anesthesia and recovery. Results showed that with both equipment the temperature was maintained approximately at 37 ± 1 ºC measured by a rectal thermal probe connected to the homoeothermic systems. But, with the new equipment, the temperature on the surface was more homogenous, decreasing the risk of accidental ear burns. The full table with the embedded equipment showed a better organized workbench and user friendly space, and has the advantage of having an easy to clean surface which may reduce contamination between surgeries.

P111 Introduction a Small Colony of Chinchillas (*Chinchilla lanigera*) in the UCLM Facility

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This work describes how we adapt our facility for the housing a small colony of chinchillas (10 males 3 months old) from a pet farm, and the basic needs for their care and husbandry. At the arrival, in all animals a complete physical and fecal examination were carried out. Daily, chinchillas were checked for assessment the food and water intake, behavior (active, curious). Twice a month were thoroughly examined to prevent more serious disorders. Animals were housed in group (4 animals/cage) in multilevel pet chinchilla cages, and were maintained in the same environmental conditions that the other rodents of the facility, although it´s very important to support a close surveillance of these conditions because they´re extremely sensitive to heat. Environmental enrichment: The cages have sufficient space to allow climb and jump. In addition we supplied dust bath (3 to 4 times weekly), and it was offered commercial small blocks of wood to assist in wearing down teeth. Chinchillas are nocturnal, herviborous and coprophagous, and need a high-fiber diet to prevent enteric problems. The diet supplied consists of well quality hay and pelleted diet in a 3:1 proportion. In summary, chinchillas can be easily housing in rodent´s facilities. They are animals of small size and very easy to handle and they have unique attributes that makes them interesting for their use in biomedical research. In contrast, for this specie doesn´t exist suppliers for laboratory animals, and they come usually from farm, and consequently, can be potential carriers of different diseases.

P112 Advancements in Animal Warming: The Modified Mini Thermacage

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There have been various warming devices on the market for the past few years. These have been introduced to aid in post-operative recovery, maintain body temperature during anesthesia and to warm animals to make bleeding from a tail vein an easier and less stressful task. This smaller footprint model was introduced into the European market a few years ago for use in cabinets, within isolators or where space is at a premium. Following positive user feedback on the original model, and along with a UK Pharmaceutical facility and a prestigious academic institute, a project was undertaken to modify the original model culminating in the new improved Mini Thermacage. It was felt that the airflow in the compartments could be made better and a number of air diffusers were considered. Feedback from customers

also suggested that there could be further refinements to the animal enclosures which have been acted upon. The new model has been trialed under test conditions and also in animal facilities and has been found to show better heat distribution, have better operator usability and provide a less stressful environment for the animals. The increasing focus on precision and also the 3Rs led to the decision to launch the improved model into the market in 2010 with an advertising campaign and trial units offered to potential clients. This poster aims to follow the process of review, updates and finally launch of the new model into the marketplace worldwide.

P113 Sources of Loud Ultrasound Noise in a Modern Laboratory Rodent Facility

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It is well known that ultrasound noise, which is audible to rodents but not to humans, can be emitted from a variety of sources in laboratory animal facilities. This is significant, since intense ultrasonic emissions could disturb or stress animals and potentially interfere with research results. Here we report on sources of loud ultrasound noise in a modern rodent facility. Acoustic emissions (0 to 150 kHz) were measured with an ultrasound detector at a 10 cm distance from potential sources and at cage (that is, animal) level, with recordings analyzed using BatSound Pro software (v3.31, Pettersson Elektronik). The most significant sources of loud ultrasonic emissions, which were within the frequency range that is most sensitive to rodents' hearing (that is, 7-50 kHz), were lights, biosafety cabinets, air-supply units to individually ventilated cages, and radios. Roof lights (depending on technology) emitted noise of up to 125 decibels (dB) in loudness (that is, sound pressure level) with values up to 105 dB at cage-level (5-130 kHz) for standard fluorescent tubes, and up to 95 dB (45-105 kHz) for dimmable lights. Air-supply units (located next to ventilated cages) emitted noise of up to 55 dB (5-80 kHz); biosafety cabinets emitted noise of up to 60 dB (2-60 kHz); and radios emitted noise of up to 95 dB (2-150 kHz). These emissions are often louder than normal mouse vocalizations (that is, approximately 20-70 dB) and could affect animal behavior. Intense ultrasound emissions are thus present in modern facilities and should be addressed during facility design and maintenance.

P114 Heterogenity of Light Intensity in a Mouse Housing Room: Consequences on the Managment of Light Sensitive Animals

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Light intensity is one of the physical parameters who can have a main effect on the physiology of the rodents, in particular on albino animals. So, we have measured the light intensity inside the cages of a room in the experimental P1 area of AniRA- PBES (Plateau de Biologie Expérimentale de la Souris). This room is equipped with conventional or IVC racks. The intensities measured are in conformity with the different recommendations with values of 325 lux about 1.0 m above the floor. The measured intensities are very heterogeneous with the cages at the top of racks receiving the maximum of light. So, it is better to house animals with retinal degeneration sensibility (albino animals) on the lowers shelves of the conventional racks or in IVC. It is also better to not modify the position of the cage on the rack to conserve a constant light intensity during the experimentation.

P115 Start-Up and Maintenance of a Mosquito Colony

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Malaria is the most prevalent parasitic vector-borne disease with 800,000 deaths annually. This human disease is transmitted by the deadly bite of the *Anopheles* spp. mosquito. The rapid emergence of resistance to available antimalarials calls for the development of new, safe, inexpensive, and orally effective medicines. In recent years, the focus of malaria efforts has moved from control to eradication. To achieve this goal, the discovery and development of new molecules that block the transmission of the disease via the mosquito vector is vital. Therefore, it is necessary to have adequate facilities to work with mosquitoes. We have designed and built and insectary in the animal facilities of our Center (by modifying previously existing animal rooms). This refurbishing is part of a project funded following a Public-Private-Partnership model in collaboration with Malaria Medicines Venture. Our goal is to provide scientific and technical leadership in transmission blocking methods for malaria in support of GSK and nonGSK MMV-funded discovery projects. In order to achieve this objective, the project team was responsible for the design and construction of the mosquito facilities with appropriate equipment to support: *The breeding and maintenance of *Anopheles* colonies. *The development of new experimental tools to evaluate transmission blocking at industrial standard level for antimalarial drug discovery. This poster describes the start-up of the facility, the necessary equipment and the breeding and maintenance of the mosquito colony.

P116 Analysis of Unit Facilities for the Optimization of Celtic Pig under an Extensive Management

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Our Association of Celtic Pig developed a novel system to take advantage of silvopastoral integrated areas of the forest in Galicia (NW Spain) with the fundamental premise to preserve the landscape quality and biologic diversity, minimizing therefore the environmental impact. The design of the experimental facility is a set formed by a closing area, equipped with automatic control systems adapted to pig livestock. Through this system the animals are learned to fed themselves. Feedstuff is essentially based on the resources of the forest and the feeding takes place in the closing area in response to a sound signal. Once the animals enter to the closing area they are automatically confined in it, but then animals have the possibility to freely enter and exit. The infrastructure facility has a feeding system fueled by a circuit of spiral tubes, in which the transport of feedstuff is powered by an engine from a silo placed outside of the closing area to the feeders. A water store provided water for drinkers by gravity. The system of opening the access door to livestock and the system to bring closer the feedstuff to the feeder system are both automated. All engines and automatisms are powered by electrical energy from solar panels. First results of this facility indicate a good adaptation of the pigs. No observations of animal behavior problems or significant changes in live weight were found.

P117 Laboratory Animal Sciences Courses Offered at the National Veterinary School of Toulouse

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The continuing education unit dedicated to the Laboratory Animal Sciences is very active at our institution. The currently provided specialized training courses are: Use and care of laboratory animals Level I (FELASA C level accreditation since 2006), Use and care of laboratory animals Level II, Necropsy, sampling and histology of laboratory animals, Fertility and behavior of laboratory animals, Normal and abnormal embryology of laboratory animals, Neuro-anatomy, neurohistology and neuro-surgery of laboratory animals, Training of ethic committees members in animal experimentation. Moreover, some additional continuing educations courses or programs were built in response to institution's specific needs: Handling, administration and sampling in laboratory animal, - Histopathology of mouse models oh human cancers, Necropsy and methodology of the macroscopic examination in rats and mice. The staff includes lecturers from both private firms and public institutes *(and from French Veterinary schools. These courses are attended by an increasing audience from various public and private French research institutes. They bring together researchers, trainees from PhD schools, technicians, executives and veterinarians from the Pharmaceutical, Chemical and Plant Health industry. An exhaustive presentation of these training courses is available on the electronical continuing education page of our institution in "Laboratory Animals" file (http://www. envt. fr/Enseignement/page2321. htm).

P118 Stereotaxic Neurosurgery in Laboratory Rodents: Handbook on Best Practices

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Techniques of stereotaxic surgery are commonly used today in research laboratories by a diversity of students, technicians and researchers. To meet the evolving requirements imposed by international legislation, and to promote the implementation of 3Rs rules (Replacement, Reduction, and Refinement) by reducing experimental error, animal morbidity and mortality, it is essential that standard technical procedures and proper conduct to follow during such complex surgeries be precisely described and respected. To this end, it is indispensable to have a comprehensive document of reference that is practical, ethically coherent and in accordance with the European Directives on laboratory animal welfare. This handbook has been conceived to respond to these needs by specifically addressing ways of conducting pre-, per- and postsurgical procedures. Along the different sections of the book, the investigatoris accompanied in a simple, systematic yet precise manner, in the practical aspects of stereotaxic neurosurgery, use of stereotaxic atlases, handling of stereotaxic frames. Supported by up-to-date reference sections and illustrations, fundamental aspects of surgery such as sterilization, asepsis and wound suturing are covered, with particular emphasis on applying and optimizing anesthesia, and instruction on anticipating, evaluating and treating animal pain and discomfort during and after surgery. The content of the book is designed as a unique and comprehensive source for all users of stereotaxic procedures, even those with limited experience, to assist them in identifying and applying optimal surgical methods, towards achieving high quality experimentation that combines reliable and reproducible results with an acute awareness of ethics and animal welfare.

P119 The EUPRIM-Net Course Series: Upgrading Personnel to Support Animal Welfare

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Nonhuman primates play an important role in biologic and biomedical research. The EU-funded European Primate Network (EUPRIM-Net) brings together nine European primate centers and is aimed at advancing knowledge and competence in biologic and biomedical research, primate keeping and breeding. For housing, breeding and experimenting with primates, the principles of Good Scientific Practice and Animal Welfare are crucial. Hence, all personnel involved in primate research are required to have a good understanding of primate biology, to ensure that animals receive adequate care under appropriate keeping conditions. Accordant conventions and recommendations have been formulated by the Council of Europe (J. EC 1986) and FELASA (Felasa, 1995; 1999). However, existing knowledge and expertise are often widely dispersed. In an effort to pool essential knowledge and to disseminate it to all staff working with primates, EUPRIM-Net has developed a series of courses for a broad international auditorium, mainly scientists, veterinarians, colony managers and students. Additional courses are addressed specifically to animal caretakers and technical staff. The intention of the course series is to spread sound knowledge and latest developements quickly across Europe to support science that meets the highest ethical standards for primate-based research. Accordingly, these courses cover a great variety of topics, related to the wellbeing of captive primates, including their general biology, behavior, husbandry, medical aspect, environmental enrichment and ethics. Furthermore,course participants can improve their interpersonal skills to communicate state-of-the-art primate-based research. The presentation gives an overview of the concept of the EUPRIM-Net course series, its contents and target audiences.

P120 Using Role Play to Debate Animal Testing: An Experience with High School Students at Barcelona Biomedical Research Park

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During scholar visits to our institution students frequently asked about and showed interest in the use of animals in biomedical research. This issue generated a long debate which was difficult to drive. For this reason we decided to design a special activity. Because the animal facility is a restricted area, we designed an alternative activity based on debate. The chosen resource was role-playing game because this type of game requires students to defend different positions and allows participants to debate and reflect on their personal opinion. It was designed based on existing european role play materials for the debate on laboratory animals use. Their content was adapted to the Catalan context and language. During the visits, the topic of animal testing was introduced with a 30 minute talk by a animal facility staff member and afterwards the role-playing game was performed. More than 180 15-18 years old students participated in the activity. We analyzed the key elements for designing a role play game, as well as its impact on student's opinions. Our preliminary results show that students actively took part in debate and made use of the new information provided by the game. Although only 23% of them altered their opinion on animal testing after the game, 70% highlighted thet the activity had enriched their point of view and given them better arguments with which to defend it. In conclusion, the role-play activity promoted critical thinking and argumentation skills, stimulated students´ participation and helped them to empathize with different viewpoints.

P121 Without Animal Suffering and Pain: Pedagogic Results of the "Surgical Techniques Applied to Experimentation Without the Use of Live Animals" during the Past 2 Years

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WASP Science develops and delivers high-quality, accredited courses and programs, initial or continuing education trainings in fulfilment of the 3Rs rule. The "Surgical Techniques Applied to Experimentation without the Use of Live Animals" is three day course and training accredited

by the *National Commission of Animal Experimentation*, approved by the *French Ministry of Agriculture and Fisheries*, and thus allows trainees to request a license to perform experiments that include surgery protocols. Under Swiss regulations, the *Association des Vétérinaires Cantonaux* validates this training as three days of professional development for study directors and experimenters. After an Introduction to experimental surgery, review of regulations and ethics, the objectives and means are acquisition of a coherent methodology for surgical asepsis, thinking and choice of methods of anesthesia and analgesia, and analysis and implementation of a surgery. The acquisition of safe and accurate gestures to avoid any risk to the practitioner and to limit the patient's trauma is based on demonstrations followed by tutored practice and individual evaluation for each exercise. Case based discussions and analysis of participants' procedures help to understand clinical follow-up, pre-, per- and post-operative care. At the end of this innovating training, 75% of the student confirm that the use of live animal is not necessary and it's grow up to 84% six months later. The training impact is good, 70% of the participant change their proceed in surgery. We hope to have the same results with speciality training like oncological surgery, initiation to acute or chronic catheterism, initiation to microsurgery and vascular microsurgery.

P122 Fulfilling the Global Needs for Training of the Animal Research Community within a Pharmaceutical Organization

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Meeting the requirements of EU Directive 2010/63/EU for training of animal research community will be a challenge for organizations within the EU. There are four major objectives that will drive the development of a globally harmonized training program within GlaxoSmithKline (GSK). The first of these objectives is verification of competency to perform procedures. This requires the development of training standards and an assessment process. The second component is delivery of continuing education for anyone working with research animals. Consideration needs to be given to the content, scope, depth and frequency of the training, recognizing that new learners may have different needs than experienced personnel. Thirdly, there needs to be a consistent and auditable method for documenting the delivery of training and assessment of competency. GSK has adapted a global learning management system that is applied across the corporation for the purpose of managing training documentation, to include providing content and storing training records. Lastly, globally available resources to allow for sharing and adaptation of material for site specific needs are required. A network of training coordinators serves to facilitate sharing of information that includes methods, ideas and newly published information for improving the global training program. Achieving these objectives will enable GSK to meet the requirements of EU Directive 2010/63/EU as well as national laws for all other countries in which the company operates animal care programs.

P123 Competency Training in the Laboratory Animal Science Environment, the Triumphs and Tribulations

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The implementation of the Directive 2010/63/EU on the protection of animals used for scientific purposes places responsibility on individual establishments to "ensure that staff are adequately educated, competent and continuously trained and that they are supervized until they have demonstrated requisite competence". Our Research Support Facility provides a full animal care and scientific service to the large scale Mouse Genetics Project and Zebra Fish Mutation Resource as well as twelve other Faculty research groups. This work is supported by a team of nearly seventy people employed in a wide range of roles spanning every aspect of laboratory animal care and welfare. Specific areas include logistics and hygiene services, animal technicians of differing experience levels, procedure and welfare specialists, scientific managers and administrators. In addition we also provide technical and procedural training for scientific staff. It is vital to the provision of consistently high animal care standards that all of these individuals receive the appropriate level of interlinking knowledge and skills to maintain competency in a dynamic environment. Prior to the implementation of the Directive 2010/63 EU we embarked on a review of our training provision and discovered that in a large, complex and diverse facility the provision and monitoring of adequate levels of education, training and competency is very challenging. Here we highlight these challenges, offer our approach to overcoming them and describe our on-going efforts to provide an effective and demonstrable program of training that can be tailored to the needs of the facility, its occupants and its users.

P124 Establishment of the Subject the Laboratory Animal in the New Degree in Veterinary Medicine at the University of Córdoba

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The EHEA, with the aim of standardizing Europe's Higher Education, has had all the university decrees modified. The University of Córdoba, with the establishment of the veterinary decree, believed on the need of incorporating a subject which could help to educate the laboratory animal specialists. To accomplish this kind of challenge the University needed, not only classrooms or laboratories, but also the Centralized Service of Laboratory Animal Resources. "The laboratory animal" is a 3 ECTS credits optional subject given during the first semester of the fifth course. It is a multidisciplinary subject, relating to the science and technology applied to the animals used for experimental purpose, where different areas of knowledge will be incorporated. We want the students to obtain the ability to resolve problems, to work within a team, and to apply their knowledge in their daily practice. They will also acquire welfare and bioethical knowledge, ability and practical know-how of veterinary principle and methodology, and also the veterinarian general skills and competences. The training of the veterinarian specialist on science and technology of laboratory animals is absolutely necessary under the new European directive 2010/63/UE on animal experimentation.

P125 A Particular Continuing Education Program: Experience of a Multiinstitution Collaboration

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Continuing education for animal technicians is becoming a must in research institutions. All animal facilities that want to follow the laboratory animal science recommendations or work under quality systems need to schedule a training program for their staff. The purpose of our presentation is to introduce our experience in organizing a long term continuing education program, through company cooperation system. Thanks to the efforts of our research institution and of the on-site animal facility management company, a biannual training program has been possible. This program included 12 monographic seminars about laboratory animals' science topics. Each session was free for all professionals of the sector, and it was announced previously. It was a successful experience for several reasons. There were more than 800 registrations. The background of the attendees was very diverse: apart from the technicians and researchers from our own institution, there were many other professionals that came from other institutions including academia, research institutions and/or Pharma companies. The surveys showed that apart from updating their knowledge, the attendees highlighted the importance of the interaction with others and

the experience sharing. It has been also a tool to motivate our technicians, giving them the opportunity for professional development and for establishing personal relationships with peers.

P126 Interactive Case Vignettes: A Teaching Model to Prepare the Next Generation of Veterinary Pathologists and Clinicians

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Computer software and telecommunication technologies use has revolutionized the academic sphere allowing students greater control and responsibility for their learning. In biomedical sciences, pathology constitutes a bridge between the basic sciences and clinical medicine in veterinary schools, medical schools and graduate pathology training programs. Restrictions in the use of nonhuman primates (NHP), in particular chimpanzees, in biomedical research are creating the need to find alternative ways to train professionals to study pathologic conditions of NHP. In an effort to fulfill this goal our institution has developed a series of simulated case presentation sessions. These include pathologist-clinician encounters through an interactive vignette model that improves student exposure to NHP diseases. Here we will present the use of interactive vignettes that use clinical data, clinician-pathologist narratives, video tutorials of whole slide scans and whole slide imaging. Each session links patient history with physical examination findings and clinical laboratory data to illustrate clinical-pathologic correlations, and provides examples of gross and microscopic pathology. After completing a questionnaire, students review virtual slides and have access to the video tutorial for additional assistance. Each case vignette is interactive and provides a complete explanation of the correct and incorrect answer choice. This model allows students to learn and grasp pathologic aspects of disease while providing a dynamic and systematic relationship between the learner and the learning environment. In addition, these pathology interactive vignettes provide flexibility, reproducibility and portability.

P127 eLearning in LAS to Meet Education and Training Requirements

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According to Directive 2010/63/EU all personnel involved in the use of animals for research purposes need to be adequately educated and trained (Article 23). An expert working group set up by the EU Commission is currently drafting learning outcomes for a modular training approach, to provide guidance to the MS in establishing in laboratory animal science (LAS) education and training and to help facilitate free movement of personnel. Online learning offers the opportunity to meet the proposed modular training concept and to help establish an EU wide harmonization in LAS education and training. In this context, vtk online is a tri-lingual (EN/FR/DE) online project to teaching LAS, featuring over 400 pages and 45 videos as well as zoom-in picture material on anatomy and histology. Topics cover legislation and ethics, husbandry, biology of different laboratory animal species, analgesia and anesthesia as well as procedures on animals. The platforms modular built allows a highly customized teaching approach which is in line with the new recommendations for LAS education and training. In addition, vtk online not only includes Multiple Choice self-assessment quizzes but online exams as well, where passing of exams can be used as proof of continuing professional development (CPD). The content can be adjusted to different user groups and study needs where institutions can upload and integrate own teaching content and/or additional information if needed. In the future, we will include online lectures and further expand the content, for example, developing modules on species such as birds, on pain assessment and others.

P128 Considerations of Age of Sexual Maturity in Cynomolgus Monkey: Correlation between Serum Hormone Levels and External Observations

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The goal of this study is to evaluate different parameters to establish the age at which the menstrual cycle commences in females and the age at which males are sexually mature before 5 years. Females: vaginal cytology, vulvar appearance and sexual hormone levels (17b estradiol and progesterone) were evaluated weekly over 3 months in 8 animals with ages between 3 and 3.5 years. The cytologic examination of the vaginal smears showed that the number of cornified cells increased towards the middle of the cycle and parabasal cells were observed mainly at the beginning of the cycle. The ratio of cornified to parabasal cells was well-correlated with blood estradiol and progesterone levels in most animals. The present study demonstrated that in some cases, sexual maturation of female begins before 3.5 years although cycles are irregulars. Males: External measurement of both testes to evaluate testicular volume and measurement of hormone levels (Testosterone) were evaluated monthly over 3 months in 31 animals with ages between 3.5, 4.5 and 5 years old. The results of this study demonstrated that there is a direct relation between animal age, hormone levels and testicle size. It was observed that some animals at 4.5 years old has sufficient hormone levels and corresponding testicle size to be sexually mature as opposed to 3.5 year old animals whose testicles were smaller and whose hormone levels were insufficient to be sexually mature.

P129 Implementation of the Directive 2010/63/UE in the Quality Management of Education and Training: Experience Feedback in a Nonhuman Primate Center

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Our company Silabe is specialized in lab services involving nonhuman primates (NHP). A team of 25 employees is in charge of the management of the 1000-NHP colony. The new directive 2010/63/UE requires that the staff involved in animal experimentation and care should be adequately educated and trained. Silabe came to fulfil the requirements of French new regulations based on its ISO9001 quality system management. This quality standard facilitated the law implementation without major disruption in the organization of the institution. Our education and training management system already includes: * Identification of each person's competences and responsibilities * On-the-job training program for new staff including sponsoring * Continuing education activities for all staff (on-the-job training, courses on specific skills, participation in LAS events…) * Overseeing and validation of the training program by the person in charge of human resources. The new regulation focuses on the designation of a qualified veterinarian, an animal-welfare body and the identification of the main responsibilities of people in charge of animal care and procedures. Internal quality tools allow the follow-up of such requirements like individual training records, skill board, and training plan. The new directive leaded us to reinforce the management of individual skills acquired by the staff all along its professional career. We then improved in 2013 our system by implementing an individual "skill notebook".

P130 Animal Attitudes and the Role of Laboratory Animal Science Education

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The education provided by our department is besides the transfer of scientific knowledge on laboratory animal science (LAS) dedicated to building the attitudes of students towards the (emotional) demands of animals. This way we aim to contribute to animal welfare by offering knowledge on the demands of animals and building critical attitudes in our students towards the use of animals. For animal attitude evaluation we conducted an anonymous survey at the beginning and at the end of our LAS course. This course follows FELASA C guidelines and is obligatory in the Netherlands for conducting animal experiments. Students enrolled in this course are mainly researchers at the onset of their animal research career in the Netherlands. We took the survey before and after the course with questions about different categories of animals; pet, pest, profit (PPP scale; Taylor et al., 2009) and laboratory. For the laboratory animal's category we distilled 9 questions from a list of 36 questions in a validation study to differentiate the most discriminating questions on attitudes towards laboratory animals. This study was conducted in 3rd year veterinary students. For the effects of the LAS course on attitudes the hypotheses are that: "The PPP scale is useful to investigate possible attitude change in the LAS course", that "Attitudes of students change within the two weeks they follow the LAS course" and that "Differences in attitudes are due to gender, upbringing and educational background". Ultimately, answers to these questions will lead to insight in and reflection on our education.

P131 A Training Program for ENT Residents in Head and Neck Surgery

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One of the most challenging goals for an ENT resident is to gain skills as a surgeon throughout his training period. The swine model is an excellent model for research of a various number of human diseases and have made important contributions to the clinic. They have anatomic and physiologic characteristics similar to the humans that make them ideal models for investigation. Therefore, we have developed a training program in head and neck surgery using a swine model. From October 2012 until January 2013 we performed 42 procedures at our institution: 6 functional neck dissections, 15 tracheostomies, 10 total laryngectomies, 2 partial laryngectomies, 2 facial nerve and parotid gland dissections, 3 thyroidectomies, 2 hemiglossectomies and 2 laryngofissures. Two residents participated in each session, following the instructions of one of the head and neck surgeons of our department. Pictures from the different procedures were taken in each session. This model allows training in the skills required for being an ENT surgeon. Besides, it provides information about the cervical anatomy of the swine model. Further studies could be performed in the future in topics such as surgical oncology, larynx transplantation or microvascular reconstruction.

P132 Promoting International Development of Advanced Training in Laboratory Animal Medicine: IACLAM Associate Membership

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The welfare of research animals, quality of scientific data, and institutional reputation significantly depend on assurances that veterinarians managing and overseeing research animal care are adequately educated and trained for this work. Recent focus group discussions conducted by the International Association of Colleges of Laboratory Animal Medicine (IACLAM) have indicated that the knowledge and experience of laboratory animal veterinarians can vary widely, and globally there is a lack of harmonization in expectations for laboratory animal veterinarian qualifications. As recently described in an article collaboratively developed by the OIE, IACLAM and ILAR, around the world, education and training for veterinarians in laboratory animal medicine ranges from applied specialty board certification to on-the-job exposure. To begin to address these national or regional training gaps and encourage development of post-graduate training programs in laboratory animal medicine with high quality and harmonized outcomes, IACLAM has developed an Associate category of membership. The purpose of this category is to support and mentor veterinarians involved with establishing nascent colleges of laboratory animal medicine. It is hoped that by providing a development framework with defined milestones for success, new colleges of laboratory animal medicine can be established around the world. The end goal of this work is to enhance the welfare of research animals and the quality of the science that is conducted using animal models. This poster will discuss these and other measures for supporting the development of training programs internationally in laboratory animal medicine.

P133 The Effects of Changes in Legislation on Staff and Researcher Training in Laboratory Animal Science in Ireland

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We present an outline on current policies surrounding both inhouse and outsourced education and training in laboratory animal science within our institution. We describe the importance of education and training, outlining the increased need for further education and training of staff, for their own benefit and to ensure compliance on regulated procedures. We describe methods used in ensuring all researchers are highly educated and trained in working in an animal unit, enabling ethical, responsible use of animals and strict adherence to the 3Rs. We give examples of how empowering education and training can be to staff, enabling them to improve their working environment and that of the animal. Within the new EU directive, we demonstrate the impact it has on animal facilities, showing the increase in paperwork and the depth of record keeping needed. We also discuss how changes to animal units affect both the staff and researchers working within the unit. We examine the procedures and documentation tput in place to record training, and assess the criteria we have for our staff and researchers attending further education and how we encourage active participation of both groups. All staff and researchers must attend training courses, be signed off for licensed procedures and we maintain a central information management database that captures the training and education records on an ongoing basis to enable easy retrieval of data during compliance inspections.

P134 Training in Animal Science? E-Learning as a New Approach

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A significant number of people joining research institutes are likely to be using animals in their research. Making sure that new animal users are properly trained is a legal requirement but above all an important refinement measure. Both teachers and trainees are usually busy people and lack of time is a constant challenge. At our institute we attempted to address this challenge by integrating an e-learning approach into our different training alternatives in Laboratory Animal Science (LAS). Using Moodle, a free source e-learning software platform – also known as a course management system – we have integrated e-learning as a complement to classroom lectures in our accredited FELASA Cat C course. E-learning was first introduced in our courses in 2010 and has now been used in four editions. We assessed participants' acceptance of the e-learning platform and level of satisfaction of its use in the LAS courses. Participants were handed questionnaires of E-learning Acceptance (QELA), a concordance Likert-type scale. The sample comprised 54 participants, 68.3% female, aged from 21 to 44 (M = 27.6; SD = 5.28)

PhD students and post-Doctoral fellows constituted, 31.7% and 13.7% of the sample, respectively. Preliminary results of E-learning Acceptance will be presented, with a particular focus on participants' perception of the platform's usability, contents, organization, time management and effectiveness in teaching practical skills. To conduct a high-quality and responsible use of animals in research, LAS course students must acquire a wide range of theoretical knowledge and develop practical skills. The advantages of this approach in LAS training will be discussed.

P135 Dealing with Emotions: How to Provide Appropriate Guidance for Laboratory Animal Professionals

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Humans and animals work very closely together in the field of laboratory animal research. During their stay in the animal facility, animal care staff and research team give the animals the best possible life by providing the finest care and surrounding the animals with kindness. However, during the term of a study, humans and animals often go through a lot together. Not surprisingly, a close bond is developing between them. It is well known that people frequently experience guild and sadness, during the course of a study and feelings of grief when a study is completed and the animals need to be euthanized. Acknowledging that these feelings exist and providing them with support is very important. If the existence of these feelings are acknowledged and addressed appropriately, people will feel validated and they're coping mechanism will be strengthened. Their ability to sustain and to form new bonds will be reinforced. Target audience: All people who work directly with laboratory animals. What will be learned: How to deal with the emotions that are with us every day when working with laboratory animals.

P136 Computerized Management of a Transgenic Facility, from the Task to Customer Communication

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To reduce mistakes due to information workflow and data registration, we developed, with the help of a software developing company, a solution to manage our transgenic facilities. This system is a web-based interface that manages all steps of the managing process, from the request of a customer through execution of the tasks by the animal care technician. Over other usual functionality, this system offers three major improvements compare to other solutions: live access to colonies data (continuous update) for the customer, ability to request on line tasks by the customer, task management for animal care technician. This task management system helps the technician all over the daily activity by prioritizing tasks, indicating all steps of the task, registering all data live, without stopping handling animals. Also, this system allows efficient and easy management of regulatory data.

P137 Emergency Solution in Biomodule Facing an Air Conditioning Supply Failure

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BioModule is a modular lab concept which allows for the rapid construction of a workspace that is able to meet and uphold stringent biocontainment and biosecurity requirements for the housing of laboratory animals. In Spanish National Cancer Research center (CNIO), BioModules are used to weekly recieve and maintain imported rodents. In July 2010 one of them suffered an air conditioning supply failure caused by a compressor breakage. The BioModule affected is used to house importing rodents before rederivation. Emergency plan included animal transfer to other concerted facility. However, transfer coordination lasted two days during which an increasing temperature inside BioModule had to be controlled. Develoment of a quick and optimal strategy which allows lowering the temperature to suitable values until animal transfer could be effectuated. Pierced hose pipes were placed in loop shape over the BioModule and continuous water supply was provided. Continuous falling water was able to refrigerate external structure, which results in a rapid descend of internal temperature. Temperature inside was equilibrated and monitored during those days until animals were transferred. One month later, compressor breakage was mended and worked correctly. This emergency solution provides a temporary and low investment manner to resolve an unexpected temperature rise in animal housing modules, where a not-controlled increase of temperature can be fatal.

P138 Implementation of New Data Management Software in a Lab Animal Facility: Experiences with Dos and Don'ts from a Veterinary Perspective

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Consistent documentation and tracebale data management in laboratory animal research is not only crucial for successful research and animal facility management but also mandatory since the implementation of the new EU directive 2010/63EU (c. f. article 30, 31, 45) in national law. With increasing numbers in individual animals, species, research and breeding projects, researchers, technicians and working groups, pure paperwork or digital island solutions without interfaces often reach objective limits. In our institution, a variety of species is used for research: Mice, rats, rabbits, frogs, goats, sheep, minipigs, occasionally dogs. Due to reconstructions, the mouse capacity (currently 8,500 animals) will be more than doubled in the near future. the infrastructure of the animal facility is used by more than 45 internal and external working groups; there are around 60 nwe projects authorizations per year including long term projects over several years, both (user and projects) with rising tendency. Purchased in 2012/07 and followed by trainings, data migration etc. a new management software (tick@lab, a-tune, Darmstadt, Germany) was implemented on site for all users in 2013/02 while freezing former analog and digital solutions. Radical system changes often require more than reaching objective limits of the old systems and workarounds if planned to be successful. to our experience, clear hierarchy of decision processes, figuring out stakeholders and convincing decision makers, fund raising, compliance of technicians, communication with researchers and continuous support from internal IT and software provider are among those that are vulnerable essentials.

P139 Another Features for the 3RRs: Assesing, Measuring and Changing the Perception of Society Towards Animal Experimentation

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As a part of the activities and social responsibilities of the University Miguel Hernandez, every year pre-university science students (16 to 18 years old, public and private colleges) have the opportunity to visit different laboratories at our Health Sciences Campus in Sant Joan d´Alacant, wich includes animal facilities and their Experimental Animal Service. As part of our Quality System and to guide these students during the visit, we wrote a "Guide to visit the SEA. Applications of 3RRs" which describes both: the concepts of the 3RRs and their application in our facilities. Several questions were measured, using a survey, just before and just after the visit, allowing us to assess the influence of the visit on three points: 3RRs knowledge, about our activities, and general animal experimentation. Our n was 85 people before and 91 after for a total of 374 visitors. Despite the fact that there is a favorable

collective to animal experimentation, as the initial note of 6.67 (1 to 10 scale) suggests, the final grade is 7.83 more than 10 percentage points. This allows us to say that with the diffusion of our activities under the 3RRs it is possible to improve the general opinion of this group, and transposing it to the general population, indicates that a greater effort to disseminate the 3RRs concept and its practice in each institution, could improve the point of view that the society has on animal experimentation. The survey questions and results are shown in the graphs shown in the poster.

P140 The Effects of Transportation on Physiology and Behavior of Rats

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Transportation of laboratory rodents unavoidably causes stress. Most laboratory animals used in research are vendor-bred and transported to research facilities, meanwhile experiencing numerous unfamiliar environmental and psychologic influences such as noises, temperature-fluctuations, handling, shaking, vibrations and smells during transportation. To obtain reliable scientific data from experiments using small laboratory animals, their physiologic status is demanded to normalize/stabilize up to a condition which can be defined as baseline. Using stressed animals is likely to result in considerable and unintended effects on research results. We investigated physiologic and behavioral parameters before and after transportation, as well as in transported and nontransported animals. Next to that, inhouse transportation and light regime reversals were studied. Parameters observed were: bodyweight, food- and waterconsumption, plasma corticosterone, glucose, creatine kinase, core body temperature, heart rate, blood pressure, locomotor activity and homecage behavior. Significantly decreased body weight, water and food intake were observed at the day of transportation in transported animals. Temperature inside transportation boxes strongly correlated with body temperature. Plasma corticosterone levels increased at least up to 16 days after transportation. Female control rats showed decreased glucose levels compared to transported females at day of transportation. Blood pressure, heart rate and activity showed gender specific effects after transportation. Grooming increased while social interactions decreased after transportation. With these studies we have demonstrated that there are long lasting, gender specific effects of transportation on physiologic and behavioral parameters and that there are additional effects of light regime reversal after transportation.

P141 Increased Sperm Cryopreservation Efficiency upon Implementing New CARD Methods at the Spanish EMMA Node

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Mouse embryo and sperm archives greatly contribute to the efficient and the adequate managing of animal facilities by allowing the safe cryopreservation of mouse models of interest in biology, biotechnology and, biomedicine. Currently, international mouse archives, such as our European platform, are focusing their efforts in sperm cryopreservation because the process requires less animals and time to effectively freeze down a mouse line. Sperm cryopreservation itself requires efficient methods for freezing down sperm, for thawing and using it for in-vitro fertilization purposes. We have successfully implemented the new cryopreservation methods devised by Naomi Nakagata's laboratory at the Center for Animal Resources and Development of the University of Kumamoto, in Japan, recently published, with most satisfactory results. The combined use of L-Gln in the CPA medium, the reducing agent MBCD in the preincubation medium and GSH in the last and most important IVF step, have boosted our cryopreservation efficiencies by, at least, a factor of 2, with regard to the C57BL/6J mouse strain, particularly difficult for cryopreservation purposes, as compared to previous common methods. Furthermore, these new methods resulted in comparable cryopreservation efficiencies for other common outbred backgrounds (that is, Hsd:ICR (CD1) and HsdWin:NMRI). In our hands, even though some logical variation in results is regularly observed, according to the genetic background and the mutation carried by each mouse line, overall, we obtained average IVF efficiencies of about 50% for fresh sperm and of about 40% for frozen sperm which have been instrumental for our routine operation as an archiving facility.

P142 A Risk Based Approach to Reducing Exposure of Staff to Laboratory Animal Allergens

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Within the research and biomedical industry one of the many risks for staff working with small laboratory animal models is possible sensitisation and development of Laboratory Animal Allergy (LAA), which can lead to occupational asthma. It is a requirement of the Control of Substances Hazardous to Health Regulations 2002 (CoSHH), the UK's enactment into law of EC Directive 98/24/EC, for an employer to prevent or adequately control exposure to any hazardous substance, which includes such animal allergens, so far as reasonably practicable, for the protection of all people on the premises. There is a lack of consensus in the literature regarding the level of animal allergens considered safe. Accordingly there must be continuous review and refinement of procedures and activities, with the aim of reducing the potential for exposure to LAA. This is achieved in part by putting in place infrastructure and environmental controls, ensuring associated equipment is regularly serviced and conducting risk assessments for all activities. Although not a requirement of CoSHH, monitoring allergen levels is central to assessing the effectiveness of the controls and refinements. This account explores the approach used at our Institute to monitor its animal facility environment allowing us to map and define the potential levels of LAA. In doing so we have developed a risk based approach to specific activities ensuring adherence to best practice and risk of allergen exposure is minimized, providing a safer working environment at all times.

P143 Management Challenges of a Complex and Continuously Growing Animal Care and Use Program

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The Department of Experimental Medicine at University of Copenhagen consists of six geographically separated animal units and the Department's constant effort to create a common team spirit, uniform work processes, and maintain all units in compliance with the AAALAC accreditation, creates a continuous demand for developing and applying tools and strategies for an efficient and transparent management of this large and complex animal care and use program. A change to IVC housing systems in all our units has created new challenges regarding health monitoring and handling of increased activity in a confined space automatically creating challenges with respect to the working environment, in particular in relation to ergonomics and allergen exposure. In addition, the ambition to operate the Department as a professional business with the researchers as our customers, which we strive to provide a world-class service, often brings challenges when the researchers' wishes collide with for example, health and safety issues, animal welfare issues, our strict subdivisions according to animal health status, or rules and regulations from the national authorities. This presentation will give an overview of how we have dealt with management challenges of our complex and continuously growing animal care and use program.

P144 Preferred Rest Area of Mice into the Cage

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If we consider the surface of the cage bedding divided into four equal rectangles, each located in the area closest to each of the four corners, the rectangle located at the right rear corner (RR) of the cage appears to be the site preferred by mice to rest. In the study we carried out 3127 observations on 107 cages in 3 different rooms. The cages contained mice of different strains and have been considered both individualized mice as groups. According to our data in 37.7% of occasions mice lie in RR area, while other three areas have a similar use, around 21% each. We have also seen that 63.6% of mice used to rest mainly a single area of the cage; 27.3% also use a second zone, although to a lesser extent; 7.8% divided its activities into three areas and only 1.3% used the four areas on a regular basis. An increased use of the whole rear area is observed as you go down in height, while the cages located in the upper shelf of rack rear area is used in 49% of cases, the percentage is on the rise as it descends up to 68% on the penultimate shelf. These percentages are more accentuated in the case of the isolated breeding males where the preference of the rear area average is 78%, reaching 100% in some of the cages. Although data presented an important statistical dispersion results were consolidated as the sample size increases.

P145 Mouse Metabolic Phenotyping Platform at University of Turku

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Obesity and related metabolic diseases are increasing at an alarming rate worldwide, and thus animal models are needed as translational models for human disease. Obesity is a result of environmental (lifestyle) and generic (susceptibility) factors leading to positive energy balance where excess energy is stored as triglycerides in adipose tissue. Obesity predisposes to metabolic diseases such as type 2 diabetes (T2D) and nonalcoholic fatty liver (NASH). In order to fully characterize a translational animal model in obesity research, a wide spectrum of equipment and experiments are needed. At our institution, we have a fully equipped mouse metabolic phenotyping platform available to conduct in vivo experiments and analytical assays to characterize the metabolic phenotype of GM-mouse models or to evaluate drug responses. The platform includes (1) EchoMRI-700 Body Composition analyzer, (2) indirect calorimetry system, (3) wireless mouse running wheels, (4) PhysioTel telemetry radiotransmitters, (5) exploratory locomotor activity system, (6) refrigerated fraction collection system metabolic cages, (7) glucose and insulin tolerance tests (oral, IP and IV), (8) steady-state glucose and acute glucose-stimulated insulin analyses, (9) in vivo and ex vivo lipolysis studies, (10) serum lipid, adipokine and insulin profile analyses, (11) full body necropsy and (12) fatty liver analyses. We master surgical techniques and have valid licenses to conduct the animal work. In this presentation, we will present latest data from our GM-mouse models of obesity and T2D to show how this platform can be exploited in translational research.

P146 Environmental Impact of an Animal Facility: Life Cycle Assessment of the EPFL Mouse Facility

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In the context of the EPFL sustainable campus, the regular monitoring of energy data showed an important increase after the opening of the new Life Sciences building in 2008, with a consequently increase of the impacts on climate change. As this effect is probably due to the large energy consumption of the animal facility, we conducted a life cycle analysis in collaboration with Quantis (www.quantis-intl.ch). The aim of this study is to analyze further the environmental impacts of the EPFL mouse animal facility. The analysis included the following activities: Administration and back office, Mice husbandry, Cages and racks washing, Ventilation, Import and export of animals, Scientific procedures, Waste management. For all the activities, the energy expenditure, the infrastructures and the goods consumption were considered. These data were combined with the worldwide recognized LCI database ecoinvent (v2.2). Impacts on climate change, human health and ecosystems quality were assessed, using IMPACT 2002+ LCIA method. The results show that cages and racks washing is the main contributor to the impacts on climate change and on human health, mainly due to the steam produced by gas for autoclaving and water heating. If mice husbandry is the second contributor for those indicators, it is the main cause of the impacts on ecosystems quality, mainly due to cereals cultivation for mice feeding. The analysis will allow giving some recommendations for improving the environmental performances of the animal facilities.

P147 Implementation Good Laboratory Practice at Animal Facilities of Surgery Center of Minimally Invasive Jesus Usón

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Nowadays there are many internationally recognized quality certification systems, for the management of research facilities working on experimental animal model. Amongst these we can highlight UNE EN 9001 standard, Good Laboratory Practice (GLP), and Accreditation of Laboratory Animal Care International (AAALAC). The Animal Housing facilities at our Institution are managed since 2002 under UNE EN 9001 standards. This system is based on the process and work flow quality improvement of the Animal Housing Service. In 2011, we implemented all necessary procedures for the fulfillment of GPL principles, in order to carry out studies which include in vivo Toxicity, Pharmacodynamics and Tolerance, certified by the Spanish Agency for Medicines and Health Products (AEMPS). The purpose is to establish quality standards in all preclinical phases, allowing for the retrospective evaluation of these studies. Our Institution has carried out 14 specific procedures for Animal Housing Service which, along with other general applicable procedures, constitutes an essential tool which permits control over the animals' health and welfare incidences, as well as it allows for its solution. Since GPL implementation, an increase on incidences has been observed in comparison with the previous period, reflecting an improvement in the control of the animals' health and welfare.

P148 Cryopreservation of Laboratory Rodents as Backup in Case of Disaster

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Cryopreservation of embryos and gametes of laboratory rodents is a tool to guard against loosing valuable animal models, which can be lost due to the loss of genetic authenticity, inbreeding depression, or infections. Also conceivable are failures of facility equipment and environmental disasters. In addition to prophylaxis against loss, cryopreservation of strains that are not under actual scientific use frees up space and reduces shelf costs for maintaining a vital colony. Acceptable cryopreservation methods have to fulfill following requirements: a) it has to be reliable and validated for the respective samples, b) the revitalization ability of the frozen cargo has to be tested, c) samples of one strain should be stored at two different places, d) permanent cooling has to be guaranteed at any time. It is strongly recommended to establish a genetic back up for every valuable and unique strain at least. In our institution three options/methods have gained acceptance:

embryo-freezing, sperm-freezing, and freezing of ovaries. The decision for one option depends on the availability of donor animals, availability of time, and genetic requirements. Another requirement for a well-functioning cryobank are methods for assisted reproduction such as embryo-transfer and in vitro fertilization. Today, we are looking back to a more than 30 years of experience in cryobanking and we can show the feasibility of the different options to store and save valuable mouse, rat, and guinea pig models.

P149 Impact of Automation on Cage Wash Facility Operations

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Many institutions face ever-increasing rodent populations in their facilities. At the same time, the number of animal care staff multiplies proportionately. As the number of cages reaches a critical point, facility directors must decide how to streamline manual processes in order to continue to provide the same level of care without a large expansion of the animal care and cage wash staff. With the introduction of individually ventilated cages and automatic rodent cage watering systems, a greater number of cages can be handled by the same number of care staff since the amount of time needed to attend to each cage decreases. Likewise, automation in the cage wash facility has been touted to be the solution to some of the ergonomic and logistical issues surrounding the processing of the large number of dirty cages generated in facilities with high rodent populations. To assist with the job of scraping and dumping over 20,000 cages each week, we installed a robot on the dirty side of cage wash in two facilities in 2012. An assessment was performed to determine the impact of the robot on cage wash operations. Metrics measured included number of cages processed, cage wash downtime and ergonomic benefits. Although the number of cages processed increased somewhat, cage wash downtime also increased. The greatest benefit to using automation was found to be the decrease in the cage wash workers' fatigue and physical injury. These important factors must be considered when determining whether to automate in the cage wash facility.

P150 Implementation of Quality Management System in a Conventional Breeding Animal Facility

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Quality Management System (QMS) are rules and principles related to quality in the day-to-day in the organizations. The aim of this study is to report the implementation of the QMS in a conventional breeding animal facility to obtain continuous improvement in the production process. The following steps were taken to implement the system: awareness of the institution top management, definition of policy and quality objectives, critical points survey of the animal house, defining of the work structural, staff training and elaboration of documents. A situational diagnosis allowed the identification of critical points such as: inadequate control of animal production; lack of animal health and genetics monitoring program; deficiency of programs for monitoring ventilation systems, lighting, temperature, humidity and noise in the areas of breeding animal; deficiency in the routine disinfection in the animal houses areas and sterilization of materials and supplies; lack of personnel access control; absence or deficiency of equipment control; lack of animal welfare program. An action plan was established and corrective actions were taken. Factors for the success of the implementation of the QMS were the staff training program, prepared in accordance with the level of training and activities that people accomplish; and the documentation system, which allowed planning and definition of all activities. The implementation of the QMS has resulted in better planning, organization, greater involvement and understanding of work processes, improving service to users of laboratory animals, improvement of the working environment, health and welfare of both animals and people.

P151 Extended Laboratory Animal Allergens Program

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Laboratory animal allergies (LAA) are recognized as having an important impact on the health of employees working with animals. In order to determine objective criteria for protection measures, the departments of Laboratory Animal Medicine and Environmental Health & Safety departments worked jointly to make up an inventory of all situations including a risk for LAA all over the several animal facilities and started a broad range of measurements of rat and mouse urinary proteins in many different working situations. This systematic approach allowed us to revise our view on the risk levels and to determine solutions for each situation and to create a safe working environment, even for people having an allergic history. J&J policy makes wearing of respiratory protection measures mandatory when statistical analysis of personal sampling results identify that the Corporate occupational exposure level (OEL) exceeds 5 ng/m^3, but reduced the risk in the vast majority of the situations under engineering control. All kind of activities in the animal facilities were screened through personal measurements and results helped to determine which engineering controls should be installed and/or which personal protection equipment should be worn. Measurements were done in animal housing and procedure, washing areas, cage changing areas, animal reception areas, corridors, writing spaces. This common approach allowed us to demonstrate objectively the risks for LAA, the measures to be taken and more importantly to make staff confident about our well designed risk based protection program. We would like to share the lessons learned with other animal research facilities.

P152 Withdrawn

P153 Pig Jejunal Explants: An Ex Vivo Model Allowing Interaction Analysis of Co-Occuring Mycotoxins on the Digestive Barrier

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Deoxynivalenol (DON) and nivalenol (NIV) are type B trichothecenes mycotoxins produced by Fusarium species, naturally co-occuring in food and feed. The gastrointestinal tract represents the first barrier as well as the first target for these toxicants, as shown for DON (Pinton et al, 2009, 2012). The aim of the present study was to characterize the effects of DON and NIV, alone or in combination, on the intestinal tissue of pig, the most sensitive animal species. Crossbreed weanling piglets of 4 to 5 week-old (n = 6) were used for explanting jejunal tissue. Explants were exposed to DON, NIV, and the mixture DON+NIV (1:1) for 4 hours, at 0.1 to 30 µM for each mycotoxin or for the mixture 1:1. Mucosal lesions were assessed by using histopathological scores. The individual treatment with the mycotoxins DON and NIV resulted in a significant decrease of the histopathological score from doses of 10µM and 1µM, respectively. The main morphologic and lesional changes were flattening of epithelial cells, villi fusion, apical denudation of villi with the highest dose of NIV, villi with absence of epithelia were observed. The interaction effects were evaluated by the isobolgrammethod. The DON+NIV combination demonstrated synergism for IC50 whereas antagonism was observed at the lower doses. Taken together, the present data provide

strong evidence that NIV and DON mycotoxins alone or in combinations at low exposure alter the intestinal morphology. Histopathology and immunohistochemistry investigations on pig explants will allow assessing the digestive barrier alterations following toxins exposure.

P154 Refining an Experimental Model of Osteomyelitis in Rabbits

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This work presents the results of a rabbit experimental model of human acute and chronic osteomyelitis. The model used noncolonized cotton meshes as foreign bodies which were inserted into the tibia of white New Zealand rabbits together with 0.2 mL *Staphylococcus aureus* ATCC 6538 suspension. Two concentrations of microbial suspension were used: 5 × 106 and 5 × 108 CFU/ml in two distinct groups of 10 and 15 rabbits, respectively. A separate group of 10 rabbits had no foreign body, just the pathogen in the lower concentration. No sclerosing agents were used. The rabbits of the group inoculated with the higher concentration of microbial solution developed osteomyelitis earlier than the other groups, but only 20% survived until the 15th day when it was decided to be euthanized for histologic investigation. An overdose of anesthetic was used. In the other two groups of rabbits with the lower pathogen concentration suspension 2, 3 and 4 rabbits were euthanized from each group on day 30, 45, and 60, respectively. In these rabbits, acute osteomyelitis was installed on day 30 in all studied rabbits in both groups and the chronic disease on day 45 and 60 for the group with meshes and the one without, respectively. The survival rate was 90% in the group where meshes were used and 80% in the other group. The results have shown that a simple and reliable model of acute and chronic osteomyelitis can be obtained using this model with cotton mesh foreign bodies.

P155 Different Strain-Related Pathology Pattern for In Vivo Chemically Induced Carcinogenesis

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Animal model of cancer is still a mandatory tool in cancer research. Acknowledging the differences in different animal strains we have generated in three different mouse strains chemically induced skin cancer using 7, 12-dimethylbenz [α] anthracene and phorbol-12-myristate-13-acetate. In C57Black strain carcinogenesis was induced in one stage while in CD1 and CD1-Foxn 1nu strains the procedure was applied in two stages. Although it is known that C57Black mouse strain has lower susceptibility to develop skin lesions, we have obtained a cutaneous carcinoma after a longer period of time from application, namely after approximately 5-6 months. Skin lesions were seen in CD1-Foxn 1nu mice, and in CD1 strain. Out of all CD1 strain animals, the tumor development was registered in 100% percent of mice. In this case the tumor appeared clinically detectable as early as 5-6 weeks after the initial application. The immunohistochemistry of the chemically induced skin lesion induced in CD1 strain depicted a week differentiated carcinoma. Moreover from the C57Black mouse strain a cell culture was initiated from the skin tumor. A clone of tumor cells was established and using this tumor cells, skin carcinoma at origin, we have established an in vivo mouse model of skin carcinoma in C57Black mouse strain. Thus using an established carcinoma cell culture we were able to have in a resistant mouse strain a model of cutaneous carcinogenesis. Therefore different strains of mice have completely particular chemical-induced carcinogenesis, in terms of tumor type and development duration.

P156 IRTA-METAPIGS Research and Development Collaborative Plantform: A Range of Services for Experimental Studies in the Food, Pharmaceutical and Medical Industry and Academia Using Pigs as Model Organism

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Recent advances support the convenience of pig models for studying genetic, digestive, immunologic, and physiologic disorders to carry out pre-clinical studies and establish the relationship between genes, nutrition, functionality and metabolism to develop pharmacological and nutritional human therapies. Production, feeding, management and interventions in pigs are complex and require appropriate equipments, facilities and specialized personnel. The IRTA- Metapigs Collaborative Platform (IMCP) is created to support research and development that uses pigs as biomedical model and involves several scientific research groups specialized in nutrition, management, reproduction, production, meat and carcass quality, genomics, functionality, food security, welfare and animal health and offers experimental facilities, equipment and trained personnel. IMCP can use different pig populations, genetically characterized with full breeding control, produce feed mill and powdered milk specific feeding formulas, maintain the animals in experimental unitswith environmental and behavioral monitoring protocols under welfare and veterinary health accredited personel. Specialized scientific and technical staff could obtain phenotypic information (as individual feed intake and computer tomography body composition image analysis) and biologic samples (blood, urine, faeces) in live animals. In addition, necropsiesfrom different tissues, fluids and organs obtained in the experimentalabattoirand dissection room allows to perform physical, chemical, microbiological, genomic, gene expression, proteomic or metabolomic studies

P157 Pregnancy in Postpartum Estrus Induces Inflammatory Milk Production and Catagen Specific Pup Skin Inflammation in Interleukin-10 Deficient Mice

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There were some cases of pup alopecia, and interleukin 10 deficient mice showed high incidence than other strains. The objective of this study was elucidating of maternal factors for inflammatory milk production and characterization of pup alopecia. To do this, we conducted histologic examination, fostering test, qPCR, flowcytometry analysis, analysis for breeding record and LC/MS. Maternal stress such as pregnancy in postpartum estrus (PPE) might induced untimely mammary gland involution and inflammatory milk production in B6. IL-10-/-. There were any different mass in inflammatory milk, but different ionization was detected. Inflammatory milk directly induced catagen stage specific skin inflammation and hair breaking. Histologically, hypertropy of outer root sheath (ORS) and macrophage/neutrophil infiltration were typical. Inflammatory milk affected ORS of pup skin, and inflammation began with macrophage and neutrophil recruitment. New hair follicle developed next anagen stage, and this indicated pup skin inflammation

didn't destroy hair stem cell but only induced broken hair. Inflammatory milk production has relation with maternal genetic factor, and this inflammatory milk was toxic enough to influence to wild type mouse. Interleukin 10 might be important for preventing untimely mammary gland involution. This was first report for catagen specific skin inflammation and alopecia in mouse. This alopecia was recoverable model and could be used for alopecia and skin inflammation animal model.

P158 Pig as Research Models of Human Obesity: Fat Depots Evaluation in Growing Pigs with Computed Tomography

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Pig is a model in biomedical human obesity research because of its metabolic similarities. The aim of the work was to determine the relationship between fat depots in computed tomography (CT) cross-sectional images taken in live pigs at different weights in the abdomen region and fat depots in all the body of the animal. For these purpose 40 pigs were CT-scanned (13 at 30 kg, and 9 at 70, 100 and 120 kg) using a CT General Electric HiSpeed Zx/i. Three images were taken at third and fourth caudal rib (34LR), last rib (LR) and second lumbar vertebrae (2VL). After scanning pigs were slaughtered and fully dissected to obtain the weight of the flare fat, total subcutaneous fat and total intermuscular fat. From the images subcutaneous, intraabdominal and flare fat areas were determined. The highest correlations (0.96 to 0.98) were found between total fat of the carcass and subcutaneous fat measured at the different anatomic positions in 100 kg pigs, followed by 70 kg (0.81 to 0.89) and 120 kg (0.73 and 0.85) pigs. Correlations between fat depots in 30 kg pigs were very low (–0.11 to 0.19). Subcutaneous and flare fat areas increased ($P < 0.05$) with the weight of the animals. Intraabdominal fat was not significantly different ($P > 0.05$) between 100 and 120 kg animals in any of the region measured. Limited number of CT cross-sectional images allows estimate fat depots distribution in all the body of live pig from 70 to 120 kg, and this can be used in obesity and nutritional studies.

P159 Lymphatic Duct Cannulation in Awake-Unrestrained Rats

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Standard procedures for intestinal lymph collection involve surgery for the cannulation of the lymphatic duct and continuous drainage of the lymph in anesthetized or restrained animals. This protocol requires also a cannula in the duodenum for fluid infusion to maintain hydratation and to stimulate lymph production. If anesthesia interferes with the objective of the study, the animals have to be restrained for more than 24 hours and sacrificed in 48 hours. We describe a technique for cannulation of the thoracic lymph duct in rats which allows in vivo sampling of intestinal lymph in unrestrained, awake, and ad libitum-fed animals. Under isofluorane anesthesia, the lymphatic duct was cannulated with heparinized soft vinyl tubing (0.5 mm ID and 0.8 mm OD); a drop of tissue adhesive was used to secure it. The cannula was protected with a spring coil (40 cm long approx.). The incision was sutured and the beginning of the spring was positioned under the skin. Analgesia was provided locally with bupivacaine before incision and with intraperitoneal buprenorphine postsurgery. The animals were recovered from anesthesia and housed in round-wire bottom cages with free movement and access to food and water. The spring was positioned through the bottom of the cage and the lymph was allowed to flow by gravity. With this new technique the lymphatic absorption can be studied reducing the stress of classic restraining methods. Moreover, the animals do not need to be sacrificed because the cannula can be cut and sealed and the internal lymphatic flow recovered.

P160 Development of an Experimental Model of Hypoxia-Ischemia in Adult Mice

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A great deal of research is now being pursued in order to develop new therapies for brain injury and the recovery of brain function. The aim of this study was to develop a hypoxia-ischemia model of brain damage in adult mice showing low mortality rates and low variability in the extent of the lesion. C57BL/6J mice were anesthetized and under sterile conditions, the left common artery was isolated and ligated. The surgery was completed in 15 min. After recovery mice were placed in a hypoxia chamber according to the following experimental conditions: (1) 8% oxygen for 20 min, (2) 8% oxygen for 45 min, (3) 10% oxygen for 45 min, (4) 10% oxygen for 60 min at a constant temperature of 37ºC ± 0.5. Twenty-four hours later, mice were tested on several behavioral paradigms (the rotarod, the object recognition, the open field, and the Irwin tests) in order to evaluate motor and cognitive deficits. Immediately afetr testing, animal were anesthetized and sacrificed by intracardiac perfusion and their brains removed and stored for histologic assessment. The results showed that the time of hypoxia and the amount of oxygen influenced both the size of the lesion and the behavior of mice. Histologic analysis showed loss of neurons and astrocyte reactivity in hippocampus in lesioned mice with respect to sham operated control mice in all groups. The optimal experimental conditions were 10% oxygen for 60 min since it produced the greatest deficits in behavioral tests, and low mortality rates.

P161 ENU Mutagenesis Derived Asgr1Mhdabap005 Mice as a Model for Human Ideopathic Hyperphosphatasemia Showing Additional Metabolite Alterations

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Within our genome-wide large-scale ENU mutagenesis project on C3HeB/FeJ mice we obtained a mouse model carrying a biallelic missense mutation (c.815A>G, p. Tyr272Cys) in the Asgr1 gene. The mice were originally phenotyped by significantly elevated activities of total alkaline phosphatase (ALP) reaching an almost threefold increase of wild type values in homozygous mice. Following heating of the plasma the enzyme activities remained elevated suggesting that the intestinal isoform is leading to the increased ALP values. No gene effects have been reported for Asgr1 mutations in human and mice so far and Asgr1-/- knockout mice appeared phenotypically normal. Due to the so far known function of ASGR1 as it is for example, clearance of glycoproteins from serum after desialysation we performed the screening for targeted metabolomics of 190 parameters in plasma to study any supposed effects of our loss-of-function mutation on metabolism. Asgr1 mutations may be responsible for observed human hyperphosphatasemia of unknown reason. Further, we could demonstrate some amino acid, glycerophospholipid and acylcarnithine alterations in mutant mice suggesting that the mutation has a so far unknown impact on metabolism, supported by the observation that in old male mice plasma glucose levels were decreased. We are planning high carbohydrate diet challenging feeding experiments combined with glucose and insulin tolerance and non-targeted metabolomic tests to analyze if lifestyle may trigger stronger metabolic effects of Asgr1 mutation.

P162 Strategies to Improve 17β-Estradiol Mediated Side Effects in Immunodeficient Nude Mice Bearing Estrogen-Dependent Patient-

Derived Mammary Tumor Xenografts

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Estrogen substitution is a common procedure in preclinical experimental settings where potential anti-cancer drugs against estrogen-dependent breast cancer are investigated. For this purpose, patient-derived mammary tumor xenografts can be implanted subcutaneously together with 17ß-estradiol releasing pellets in immunodeficient nude mice. The estrogen substitution is accompanied by side effects especially affecting the urogenital tract of the mice which can significantly impair animal wellbeing. In this study, we describe estrogen substitution related side effects in nude mice in detail and investigate different approaches to overcome them. Implantation of 17ß-estradiol pellets of two different doses of estrogen did not alter the growth pattern of a selected patient-derived tumor model but also did not lead to significant improvement of side effects. In a series of pathologic investigations, we found that hypertrophy of the cervix might mechanically contribute to severe urinary retention observed in estrogen-substituted female mice. When using castrated male mice, a trend towards improved survival in comparison to female mice was observed when using the low dose pellets, however, the incidence of side effects was not significantly lowered. We conclude that further improvement of this experimental approach is necessary to establish a predictive preclinical model for estrogen-dependent mammary cancer with acceptable impact on animal wellbeing.

P163 Study of the Antitumor Effect of Aziridinylbenzoquinones in Mouse Hepatoma Cells

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Mouse models that recapitulate human cancers are valuable resources for the research. Although the cytotoxic efficacy of quinones has been studied extensively, the molecular mechanisms underlying this activity have not yet been fully elucidated. The aim of this work was to compare cytotoxic activity of two aziridinylbenzoquinones as well to study their molecular mode of action in mouse hepatoma (MH-22A) cells. In our investigation, the cells were treated with different concentrations of 2,5-diaziridinyl-3-(hydroxymethyl)-6-methyl-1,4-benzoquinone (RH1) or 2,5-diaziridinyl-3,6-dimethyl-1,4-benzoquinone (MeDZQ), the effect was registered as the number of residual viable cells. After, the cell death manner was evaluated. To determine the possible mechanisms of the action of aziridinylbenzoquinones, the effect of DT-diaphorase inhibitor dicoumarol, antioxidant *N,N′*-diphenyl-*p*-phenylenediamine (DPPD) and JNK inhibitor SP600125 were tested. Similarly, the expression and activation of main molecules in MAPK cell signaling pathway was studied. We have found that the concentrations of quinones that killed the half of tested cells were equal to 0.08 μM (RH1), and 0.31 μM (MeDZQ), which were lower than that of a model aziridinyl-unsubstituted quinone, duroquinone. Dicoumarol and DPPD protected the cells against the action of RH1, which demonstrated the involvement of DT-diaphorase and oxidative stress, respectively. On the other hand, SP600125 enhanced the cytotoxicity of aziridinylbenzoquinones. Taken together with the effects of SP600125 on the expression of main JNK signaling molecules, it shows that JNK signaling pathway is responible for cell survival after the treatment with RH1 or MeDZQ. The study was supported by Scientific Council of Lithuania (Project of the Global Grant Measure No. VP13,1-SMM-07-K01-103).

P164 Long-Term Consequences of Postnatal Exposure to Lipopolysaccharide or *Toxoplasma gondii*: Effect on Rat Behavior in Animal Model of Schizophrenia

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Many studies indicate an association between the incidence of environmental insults (such as inflammation) during a vulnerable developmental period of the CNS and an increased risk of the development of schizophrenia in adulthood. To simulate the effects of infectious processes on the development of the brain and determine their postnatal consequences, this study was aimed at describing the behavioral alterations in rats exposed to bacterial (LPS) components or the protozoan parasite *Toxoplasma gondii*. It is known that there is a higher *T. gondii* seroprevalence in schizophrenia patients than in the general population. The young male rats (PD 5 - 9) were injected (IP) with LPS (2 mg/kg) or with *T. gondii* (genotype II) tissue cysts (PND 21, IP). Behavioral alterations (locomotor activity, PPI) were evaluated in full adult animals (PD 90 - 100), respectively. Both LPS-treated animals and *T. gondii*-infected rats had unchanged total locomotor activity during the 30-min test, however, hyperactivity induced by acute administration of dizocilpine/MK-801 (that is, NMDAR antagonist) was documented in both models. LPS-treated animals displayed a significantly higher acoustic startle reaction. A small impairment of PPI was documented in *T. gondii*-positive male rats. These findings suggest that neonatal activation by bacterial infectious agents or by the manipulatory parasite *T.gondii* may have long-lasting effects on the future development of the behavioral phenotype and in combination with other immunologic and neurobiochemical approaches can provide novel insights into the pathophysiology of certain neuropsychiatric diseases, such as schizophrenia. This work was supported by IGA MH CR grant No. NT/13843-4.

P165 The Zebrafish: A New Model to Study Dyskeratosis Congenita

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Classic dyskeratosis congenita (DC) is a multisystem disorder characterized by the mucocutaneous triad of abnormal skin pigmentation, nail dystrophy and mucosal leucoplakia. Bone marrow failure is the principal cause of early mortality with an additional predisposition to malignancy and fatal pulmonary complications. Mutations in six genes have been identified. The products of these genes encode components that are critical for telomere maintenance, either because they are core constituents of telomerase, or are part of the shelterin complex that protects the chromosome end. It has been described that telomeres and telomerase play a critical role in the regulation of the replicative lifespan of cells, providing a potential mechanism which explains the immune abnormalities in DC. Accurate modelling of human diseases is a major goal of contemporary medical research. However, mice with extensively shortened telomeres due to telomerase deficiency do not develop progressive bone marrow failure, the hallmark of DC. In this work, we have studied the role of telomerase in innate immunity using the zebrafish as a model, focusing on development and function of neutrophils: key players in inflammation having a central role in human immunologic disorders such as DC. Knockdown of the catalytic subunits (TERT) or the RNA template (TR) of telomerase with morpholinos resulted in impaired development and function of neutrophils, in combination with increased viral susceptibility and telomere length shortening. Our results suggest that zebrafish could be a good model to study DC because they recapitulate the key DC phenotype of defective myelopoiesis.

P166 Use of Mouse Model with Upregulated GluN2B Subunits in Hippocampus for the Study of the Role of NMDA Receptors in Aging Rodents

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In humans, memory is one of the first cognitive functions impaired by ageing. The identification of the causes of age-related memory declines is necessary to propose treatments, to prevent memory loss while aging, and to delay some neurodegenerative disease symptoms such as Alzheimer disease. The NMDA receptor, a type of glutamate receptor, has been shown to be important in learning and memory. This study aimed to determine whether increasing the expression of the GluN2B subunit of the NMDA receptor in aged rodent hippocampus is beneficial to memory performance. Both young and old C57BL/6 mice were injected with the use of stereotaxic surgery with a replication-deficient Adenovirus type 5 vector expressing either the GluN2B subunit and Green Fluorescent Protein (GFP) cDNAs, or the cDNA GFP gene. Both GFP and GluN2B subunit expression were driven by a cytomegalovirus (CMV) promoter. Both reference memory and cognitive flexibility were tested by using the Morris water maze for four consecutive days. Injection of GluN2B subunit cDNA did not improve flexibility significantly in either young or old mice. However old GluN2B up-regulated mice showed a significant improvement of reference memory on the first day, but not on the second or third day. These results suggest that enhancing GluN2B subunit expression in the hippocampus can improve memory during initial acquisition, but not across learning. These results may have implications for the memory declines experienced in normal ageing and in Alzheimer disease.

P167 Generation of a New and Novel Double Knockout Strain of Mice via Cross-Breeding of 2 Single Knockout Lines

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Tachykinin 1 (PPTA) and Neurokinin receptor 1 (NK1R) knockout mouse strains are well documented and have many phenotypes in common such as lowered anxiety levels, lowered aggression, higher thresholds to pain and resistance to seizure. One of the main products of the PPTA gene is the protein Substance P (SP) which is involved in a multitude of processes including pain, the inflammatory response and immune functions. The NK1R is the preferred receptor through which SP elicits its control. We generated a "double knockout" strain via cross breeding of the individual Tac1 and NK1 knockouts resulting in a new and novel knockout strain that had a deletion of both the PPTA and NK1R genes. This knockout mouse model was unique in that the preferred pathway of action for SP had been deleted. All of the strains, including the wild type background strain C57BL6/J, were subjected to behavioral testing for anxiety and for stress induced depression. They were then subjected to a pharmacological experiment using the neurotoxicant Kainic acid (KA) which is frequently used as a chemical convulsant in epilepsy studies. Surprisingly, the double knockout showed a unique and novel phenotype which was opposite to its single knockout contributors with a statistically significant susceptibility to stress induced depression ($P \leq 0.01$) and a pre-disposition to chemically induced seizure ($P \leq 0.05$) compared to wild type controls. This paper will cover the cross breeding of individual strains, the implications this will have for the 3Rs, welfare considerations and how behavioral testing can be used for both scientific and welfare evaluation purposes.

P168 Neuropeptide Y Overexpressing Mice in Diet and Low-Dose Streptozotosin-Induced-Type 2 Diabetes Model

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Human studies suggest that increased neuropeptide Y (NPY) predispose diabetic patients to vascular diseases. To study this in mice, type 2 diabetes (T2D) was induced in mice overexpressing NPY in noradrenergic neurons (OE-NPY^{DBH}), that spontaneously develop a pre-diabetic stage with age. Mice were subjected to Western-type diet combined with low dose streptozotosin (3 × 40 mg/kg, IP), which decreases insulin secretion by destroying pancreatic β-cells. To maintain hyperglycemia a single dose of streptozotosin was re-administered every 4 weeks. Weight gain, fasting glucose and feeding were followed weekly. Body composition, glucose- (GTT) and insulin tolerance tests (ITT), and echocardiography were assayed in vivo before and after treatment, and vascular function ex vivo after sacrifice. OE-NPY^{DBH} mice did not differ from their wildtype littermates in weight gain or fat mass. Fasting glucose and AUC of GTT were increased ($P < 0.05$ and $P < 0.01$, respectively) in OE-NPY^{DBH} mice, but no difference between genotypes was seen in ITT. Changes in myocardial thickness or cardiac ejection fraction were not detected. However, diet and streptozotosin impaired vascular function in both genotypes. Contribution of nitric oxide (NO) to acetyl choline (ACh)-induced relaxation was attenuated even further in the OE-NPY^{DBH} aorta. Endothelium-dependent relaxation was compensated by other mechanisms since no difference was observed in ACh-induced relaxations without NO synthase-inhibition. Furthermore, response of OE-NPY^{DBH} mesenteric resistance arteries to phenylephrine was decreased. In this study, we were able to induce a long-term T2D in mice with Western-type diet and low dose streptozotosin, and showed the co-effect of diabetes and NPY overexpression to vascular dysfunction.

P169 Obesity in Mice Overexpressing Neuropeptide Y in Noradrenergic Neurons

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Neuropeptide Y (NPY) is the most potent feeding stimulative peptide, which also decreases energy expenditure and increases energy storage thus inducing obesity. We have previously created a transgenic mouse overexpressing NPY (OE-NPY) in the sympathetic nervous system and brain noradrenergic neurons. We studied homozygous OE-NPY and wildtype (WT) mice at the ages of 2, 4 and 7 months. We measured weight gain, food consumption, energy expenditure (indirect calorimetry), locomotor activity (photo-beam device) and body composition (Echo-MRI). In addition, we performed intraperitoneal glucose (1 g/kg) and insulin (1.0 IU/kg) tolerance tests and measured adipose tissue weight, liver triglyceride content, serum insulin, triglyceride, free fatty acid (FFA) and total cholesterol levels after sacrifice. Homozygous OE-NPY mice showed significantly increased body weight at the age of 4 months although body fat mass was increased already in 2-month-old OE-NPY mice. Food intake, energy expenditure or locomotor activity did not differ between genotypes. OE-NPY mice showed increased brown adipose tissue weight with WAT-like morphology (H&E-staining). Furthermore, liver triglyceride content was increased with hepatic steatosis (Oil-red-O staining). Serum triglyceride and FFA levels were decreased while cholesterol level increased. Glucose and insulin tolerance impaired and fasting serum insulin increased in OE-NPY mice. In conclusion, NPY overexpression in noradrenergic neurons leads to marked obesity with findings of impaired glucose and lipid metabolism. Thus OE-NPY mouse serves as a novel animal model for the study of metabolic disorder and it can be exploited in studies testing weight losing effect of drug candidate.

P170 A Novel Tropism for Canine Oral Papillomavirus (COPV)

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Papillomavirus (PV) is a small, nonenveloped, infectious DNA virus that replicates in squamous epithelial tissue. Infection can manifest as papillomas or progress to carcinomas. Although the vaccines developed against human PV (HPV) to prevent cervical cancer were developed using the excellent model of COPV infection in purpose bred beagles, there have been no known infections with PV's in animals in the vulvovaginal area. Condyloma-like lesions in the vulvovaginal region of a canine were surgically removed and COPV was identified in frozen tissue samples. DNA was isolated and whole genome sequencing of the infectious agent was performed to determine if the COPV was related to one of four previously identified strains isolated from the oral cavity of dogs. The circular DNA was linearized and cloned into a pUC19 vector. All genomic and phylogenetic comparison was completed using software developed for this purpose. Whole viral genome isolation from the canine vulvovaginal biopsy indicated the presence of canine oral papillomavirus (COPV) with only two single nucleotide polymorphisms (SNPs) located within the E1 and NCR-2 regions. Using whole genome purification and analysis we identified a novel tropism for COPV, and the first observation of infection with this PV in the vulvovaginal area. This characteristic is shared with HPV strains 6 and 11, in that they cause lesions in both genital and oral areas. Reevaluation of the SNPs within the E1 and NCR-2 regions is in progress to further define the novel tropism of COPV and its potential as an animal model for human disease.

P171 Effect of Genetic Manipulation of Pain Pathways: Spontaneous Dermatitis-Like Skin Lesions in CD1Prrxl1 KO Mice Caused by Neuropathic Itch

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Pain remains an important health problem and animal models are an essential resource. Whether itch and pain, are mediated by distinct neural circuits has been the subject of controversy and itch has long been considered to be a sub-modality or sub-quality of pain. To evaluate how pain pathways are developed, we used CD1*Prrxl1*KO mice that display alterations in sensory afferent projections with persistent deficit in pain however in the course of maintaining breeding colonies, we observed a sporadic development of alopecia, skin lesions and a stereotyped behavior associated to an excessive grooming and increased pruritus. The pattern of grooming behavior, was registered and classified, as licking, scratching and itching. Our analysis revealed a significant difference in the time spent itching, between wild type and knockout animals (1.1 ± 0.58 compared with 196.4 ± 84.0; MW test, $P < 0.01$). The excessive grooming resulted in skin lesions in 58.82% of the CD1*Prrxl1*KO mice and started developing hair loss at 11 weeks of age, with a progressive increase until 7 months of age, followed by improvement in older animals. Significant behavioral differences were observed in pain measures with CD1*Prrxl1*KO mice presenting decreased pain sensitivity. Growth curves and health parameters were determined, no differences were observed in hemogram, total proteins and ratio albumin/globulin. Histopathological analyses showed abnormal density of hair follicles, epidermal hyperplasia, and deposition of collagen as a result of chronic itch. The etiology of the skin lesions and the behavior suggested a genetic cause associated to alterations in sensory afferent pathways, however triggered by physiologic factors associated to age.

P172 Beneficial Effect of a Plant Extract (*Amphimas pterocarpoides*) Administration on the Ovariectomized Rat Model of Osteoporosis

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Several plant extracts are consumed as alternatives to pharmaceutical interventions for their beneficial effects on bone density. The present study investigated the effect of the extract from the plant *Amphimas pterocarpoides* (AP) (Leguminosae) administered to the ovariectomized rat model of osteoporosis. Forty mature (10-month-old) female Wistar rats were randomly assigned to 3 groups: Control, ovariectomized (OVX), OVX plus AP (150 mg/mL). AP was administered in their drinking bottles immediately after OVX for 6 months. Bone density scans of the proximal tibial region using dual-energy X-ray absorptiometry were carried out at baseline, 3 and 6 months post-OVX under brief sedation. At 3 months, the median values of the % change from baseline of Control, OVX, and OVX+AP groups were 6.4, –20.5, and –4% respectively. At 6 months, the median values of the % change from baseline were 7.9, –33.7, and –9.7% respectively. All pairwise comparisons at both time points were statistically significant ($P < 0.001$, Mann–Whitney test). It is concluded that AP administration demonstrated a statistically significant beneficial effect on bone density. Its effect was not as potent as to retain bone density values similar to the estrogen-replete Control animals, but it significantly protected OVX+AP rats from the ovariectomy-induced bone loss evident in the OVX group. This plant extract merits further investigation prior to its clinical use. Acknowledgement: The present work was funded by SYNERGASIA 2009 PROGRAM. This Program is co-funded by the European Regional Development Fund and National Resources (Project code: OSTEOPRO 09SYN-13-1076)

P173 Feeding Female APOE-KO Mice a Western Diet for 16 Weeks Does Not Change Their Body Weight or Insulin Sensitivity

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In following study we investigated the glucose metabolism and aortic plaque formation in female APO E-Ko mice after 16 weeks of Western Diet (WD) with twice daily subcutaneous vehicle dosing. Female APO E-Ko mice were fed either WD ($n = 19$) or standard chow ($n = 10$) for 16 weeks. Body weight (BW) and glycated haemoglobin (HbA1c) were followed during the study. At termination an oral glucose tolerance test (OGTT) and an insulin tolerance test (ITT) were performed to assess for metabolic changes. After 16 weeks of diet regime we observed no effect on BW (WD compared with CHOW; 26.6 ± 0.9 compared with 25.4 ± 0.9 (g); P = ns) or HbA1c (WD compared with CHOW; 3.68 ± 0.05 compared with 3.72 ± 0.06 (%); P = ns). Also the OGTT and ITT both assessed as the area under curve, was not changed (OGTT: WD compared with CHOW; 974 ± 22 compared with 1028 ± 15 (mM × min) & ITT: WD compared with CHOW; 214 ± 9 compared with 219 ± 7 (mM × min); P = ns, both cases). By the en face measurement evaluating aortic plaque area, we observed a threefold higher plaque area in the WD group (WD compared with CHOW; 27.1 ± 1.0 compared with 8.8 ± 1.1 (%); $P < 0.0001$). In conclusion, 16 weeks of WD had no impact on BW, OGTT or ITT on female Apo E-Ko mice, whereas plaque formation in the WD group was markedly increased. The lack of effect on the metabolic parameters may be due to the fact that female mice on C57bl background are known to be resistant to developing the metabolic syndrome on WD but also twice daily dosing may have contributed to the lack of BW gain.

P174 Zebrafish (*Danio rerio*) Like Experimental Model to the Assessment of Endocrine Disruptors: Effects about Gills

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The release of chemical pollutants into the environment has increased in recent years. Some pollutants are called ''endocrine disruptors'' (EDs) due to their abilities to interfere with hormonal activity. Because of that it has to establish new biomarkers to evaluate the action of this compounds. An example of it is the bisphenol A (BPA) action, one of the most abundant endocrine disruptors in the environment, about gills of zebrafish. The gills, the major uptakes sites of water contaminants could be used like histopathological biomarkers of response to contaminants exposure. Thirty males' zebrafish were randomly distributed in three aquariums to establish the groups of the study: control group (n = 10), and treated groups (n = 20) exposed in water to 10 and 1000 µg/L BPA, during 14 days. Zebrafish were sacrificed by tricaine methanesulfonate dilution. Immediately after death samples were taken out and fixed for histologic processed. Histologic modifications were observed in zebrafish gills exposed to 10 µg/L, hyperaemia and oedema processes were evident in the lamella together to microhemorrhagic processes. At electronic scanning microscopy it can be observed the degradation of the lamella surface. In the zebrafish exposed to 1000 µg/L could be observed the same processed but more aggravated, including inflammatory focus presence. At electronic scanning microscopy exit of inflammatory exudative cells can be observed, and disorganization of the basal filaments and several inflammatory exudative cells. Our results demonstrate that structural and ultrastructural study of the zebrafish (*Danio rerio*) gills could report information like biomarker in the EDs exposition.

P175 Evaluation of Skin Wound Healing Kinetics in a Porcine Diabetic Dislipemia Model

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Over 60 million people live with diabetes in Europe. The pig has gained interest as a preclinical model in diabetes research because their metabolic and physiologic responses closely resemble those observed in humans. The aim of this study was to provide kinetic data from the healing of a skin wound model in diabetic-dyslipemic minipigs. The study protocol was approved by the Institutional Animal Care and Use Committee. Six adult male Gottingen minipigs were used. Three of them served as controls while in the other three diabetes was induced by Streptozotocin administration. Maintenance feed was supplemented with animal satured fat to establish dyslipidemia on the diabetic animals. Weekly monitoring of glucose, cholesterol and triglycerides were conducted during the study. After 44 weeks of stabilization, two round-shaped full-thickness skin wounds of 16.82 ± 1.93 cm^2 were created surgically on the hind legs, under general anesthesia, proper analgesia and sterile conditions. Wound surface and granulation tissue formation were assessed from photographs taken two times per week using image software. Histopathology of major organs and wound samples were performed at the end of the study, 55 weeks after the diabetes induction. The diabetic-dyslipemic induction protocol produced an intense and irreversible diabetic state and also increased cholesterol and triglycerides levels. Intial lesions of atherosclerosis were found on the studied aortas. Statistical differences on the granulation tissue formation were observed during the first 3weeks of study. Although diabetic wounds showed a delay in total healing time and a slower granulation tissue formation, healing kinetics was very similar in both groups.

P176 A New Preclinical Femoral Head Osteonecrosis Model in Sheep

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Animal models have been used as insight into the pathogenesis of osteonecrosis, even though most have failed to reproduce all stages of human disease, limiting progression in experimental treatment modalities. The purpose of this study was to create an animal model that reproduces the evolution, including all the progresión stages of femoral head osteonecrosis in a quadruped that is easily accessible like the sheep. We also wanted to evaluate the correlation between histologic and MRI changes, since MRI is the gold standard for assessing patients with suspected or established hip osteonecrosi. Osteonecrosis was achieved using an improved method of intracephalic cryogenic lesion by means of a cryoprobe and vascular ligation in 10 mature sheep. Five sheep had MRI of the afected hip at 6 weeks and then were sacrificed. The remaining five sheep had MRI at 6 and at 12 weeks and were sacrificed afterwards. Histologic findings at 6 and 12 weeks showed progression to advanced stage osteonecrosis. Hip MRI at 6 and 12 weeks showed typical osteonecrotic changes for all sheep. Our results showed good correlation between histologic changes and MRI findings. Our model creates osteonecrosis of the femoral head in a reliable and standardized way in sheep, an accesible research animal that has similar human physiologic characteristics. We continued to refine an ischemic insult with an of-the-shelf cryoprobe that adds objectivity and precision, which helped us obtain irreversible necrosis in a quadruped with concordant histologic and MRI results.

P177 A Preclinical Model of Extremity Amniotic Band Syndrome in Fetal Lamb

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Extremity amniotic band (EAB) syndrome can cause an intrauterine amputation as a result of a mechanical effect with progressive strangulation. The aim of the study was to reproduce severe forms of EAB, which result in amputation or severe lesions and to assess the use of fetal surgery of EABs with risk of amputation in the ovine fetus. Research included 3 steps: 1- Development of EAB's preclinical model with risk of amputation. Right limbs of five 60-day-gestational age fetal-sheep were ligated at infracondylar level. The limbs obtained from at term fetuses showed amputation reproducing the effect and clinicoradiological features of EAB's in human fetus. 2- Study of experimental EAB intrauterine release to assess the utility of fetal surgery. Fetuses were randomized into three groups: early-repair group (n = 5), late-repair group (n = 5) and nonrepaired group (n = 5). The limbs of the two repaired groups underwent fetal release. Nonrepaired limbs showed irreversible deformities; the repaired ones did not. Late repaired had mild residual deformities. 3- Study of experimental EAB intrauterine release through minimally invasive techniques to assess the utility fetoscopy fetal surgery of EAB. Limbs of five fetal sheep were ligated and later on, underwent fetoscopy. At term limbs showed no amputation. Intrauterine release of EAB prevents limb amputation and leads to morphofunctional recovery. Early release shows better results. EAB's Intrauterine release is feasible by fetoscopy. Three cases of human fetuses diagnosed of EAB with risk of amputation by prenatal ultrasound and MRI underwent successful intrauterine limb salvage by fetoscopy.

P178 Grape Extract Exerts a Protective Effect on Bone Loss of the Ovariectomized Rat Model of Osteoporosis

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Although plant-derived drugs have been met with skepticism by modern society, research has recently approached these compounds with new interest regarding their potential action. The aim of the present study was to investigate the impact of the grape 'Muscat Hamburg' extract on bone density of the ovariectomized rat model of osteoporosis. Thirty-nine mature (10-month-old) Wistar rats were randomly assigned into 3 groups. Group A (n = 11) sham operated, Group B (n = 13) ovariectomized and Group C (n = 15) ovariectomized receiving the extract (150 mg/kg) daily through their drinking water. Dual energy X-ray absorptiometry was performed at baseline, 3 and 6 months after ovariectomy. Two regions of interest were placed, the first comprising the whole tibia, while the second in the proximal tibial metaphysis. For the total tibia, median percentage changes from baseline in Group A were 1.49% and 2.14%, in Group B -6.02% and -10.73%, while in Group C -1.9% and -4.60%, at 3 and 6 months respectively. For the proximal tibia, median percentage changes from baseline in Group A were 6.67% and 8.34%, in Group B -21.82% and -32.13%, while in Group C -6.04% and -8.82%, at 3 and 6 months respectively. Pairwise comparison revealed highly statistical significant differences (Mann–Whitney test) between all groups. Consequently 'Muscat Hamburg' extract exerted a beneficial effect on bone loss caused by ovariectomy. The present work was funded by Thalis 2011 program. This Program is co-funded by the European Development Fund and National Resources (Project code: SERMENCO MIS 375617).

P179 A Refinement Measure in a Model of Postoperative Ileus in Rat: Comparative Effects of Buprenorphine and Meloxicam

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Legislation and international guidelines promote the use of analgesics to reduce pain and distress in animals. Nevertheless some analgesic drugs may interfere with the study thus limiting their use in some animal models. We aimed to investigate the effect of buprenorphine and meloxicam in a model of postoperative ileus. Eight to 9 week-old male Sprague–Dawley rats (300 to 360 g) underwent laparotomy (SHAM) or laparotomy plus intestinal manipulation (IM) under general anesthesia. We have previously validated this model presenting delayed gastrointestinal motility after IM. After surgery rats received a subcutaneous single-dose of either saline (nontreated rats, n = 5) or buprenorphine (0.05 mg/kg, n = 4 to 5) or meloxicam (2 mg/kg, n = 3 to 4). At 24-h intestinal transit and gastric emptying were evaluated by means of geometric center. IM delayed intestinal transit in both nontreated and buprenorphine groups (GC: 5.25 ± 0.59 and 4.75 ± 0.25) compared to SHAM rats (GC: 6.92 ± 0.33 and 6.43 ± 0.38, P = 0.0079, One Way ANOVA). In contrast, meloxicam tended to increase intestinal transit after IM (6.29 ± 0.61) compared to SHAM group (7.11 ± 0.13). IM tended to decrease gastric emptying in nontreated and buprenorphine-treated rats (P = 0.0549, one-way ANOVA). On the contrary, meloxicam prevented delayed gastric emptying in IM rats (92.74 ± 3.2%) compared to SHAM rats (95.13 ± 0.35%). We conclude that buprenorphine does not modify our model and contribute to avoid pain-related confounding factors. In contrast, meloxicam seems to prevent delayed gastric emptying and could represent a measure to shorten duration of ileus for survival intestinal surgery in rodents.

P180 Cognitive Deficits in the Down Syndrome Mouse Model Ts1Cje

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Down's syndrome (DS) is produced by the trisomy of the human chromosome 21. Several partially trisomic mouse models are available, including the Ts1Cje mice. We have recently reported that the Akt/mTOR pathway is hyperactivated in the hippocampus of Ts1Cje animals, probably affecting memory and learning, provided the crucial role this signaling cascade has in synaptic plasticity. Moreover, dysregulation of mTOR is involved in autism spectrum disorders (ASDs). We are presently characterizing the behavior of Ts1Cje mice in a classic memory test, the *object recognition*. Our preliminary results suggest that both short-term and long-term memory are impaired in Ts1Cje. Strikingly, the exploratory index of Ts1Cje mice is significantly reduced compared to wildtype animals, suggesting a lack of motivation or interest in exploring new environments. This phenotype could be related to the ASD features frequently associated to DS. Rapamycin is a FDA-approved inhibitor specific for mTOR. The ability of rapamycin to reverse the cognitive deficits observed in Ts1Cje mice is underway. Work in the lab is funded by the Ministerio de Economia y Competitividad (Instituto de Salud Carlos III, Spain; Grant PI11/00507), Junta de Andalucia (Spain; Grant P09-CTS-4610), and Fondation Jerome Lejeune (France).

P181 11β946; HSD-1 and PAI-1 Gene Expression Fructose Induced Metabolic Syndrome in Rats

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Metabolic Syndrome (MetS) is an important health issue worldwide, its prevalence is gradually increasing mainly in the developed countries. Recent studies showed that high level of fructose consumption and the dysregulation of 11β HSD-1 and PAI-1 gene expressions in MetS play an important role in the pathogenesis of MetS. The aim of this study was to investigate the effect of 20% fructose (w/v) in the development of MetS in rats by the use of the biochemical and genetics approaches. The study group of rats was composed of the MetS group (n = 10) fed with water containing 20% fructose (w/v) and the rats in control group (n = 10) were fed with water only for 15 weeks. The gene expression levels of 11β HSD-1 and PAI-1 from liver and omental adipose tissue were also investigated by QRT-PCR and biochemical measurements in both groups. The results showed that biochemical parameters (glucose, total cholesterol, LDL, HDL, VLDL, ALT, CRP and cortisol) were significantly increased in the rats of MetS group compared with those in the control group ($P < 0.05$). Furthermore, the mRNA levels of 11β HSD-1 and PAI-1 genes were also increased in the MetS group compared to control group in liver and omental adipose tissue ($P < 0.05$). These findings show that there are statistically significant differences in biochemical parameters as well as in the gene expression levels of 11β HSD-1 and PAI-1 genes in MetS group and these changes prove the contributory role of the 20% fructose consumption to the development of MetS.

P182 Unique Animal Models for Human Diseases to Promote Drug Discovery: Laboratory Animal Resource Bank at the National Institute of Biomedical Innovation (NIBIO), Japan

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Our institute was established in 2005 by the Japanese Ministry of Health, Labour and Welfare, for the purpose to contribute to the creation of the innovative pharmaceuticals and the improvement of the national health. As a variety of disease models in small laboratory animals significantly contribute to medical research and the development of pharmaceutical products, we established the Laboratory Animal Resource Bank at our institute in 2006. We have been conducting the collection, maintenance, preservation, supply and database construction of disease model animals, especially mice. At present, more than 200 mouse strains are deposited to our bank and their embryos/sperm are safely cryopreserved. In addition, we are developing animal models for human diseases focusing on rare and/or incurable diseases including inherited metabolic disorders (for example, lysosomal storage diseases), intractable nephrosis, cardiomyopathy and epilepsy. We will give an overview of our activities and the unique collection of disease model mice, which are available to researchers in all over the world. Related URLs: http://animal. nibio. go. jp/ [1] http://www. shigen. nig. ac. jp/mouse/jmsr/ [2] http://sagace. nibio. go. jp/ [3]

P183 Food Intake Measure Can Be Combined with Intravenous Dosing and Sublingual Blood Sampling without Inducing Stress in Rats

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An important early step in the discovery of new anti-obesity drug candidates is the evaluation of efficacy in rodents. The aim of this study was to combine automatic monitoring of food intake with plasma exposure measurement without inducing additional stress to rats, using liraglutide as model compound. SPRD rats were group housed and had ad libitum access to food and water during the study. The HM-2 food intake system automatically measures the food and water intake continuously identifying each rat via a chip implanted in the upper part of the neck. The system records each feeding event and the amount and duration of the meal. Data can be collected without disturbing the animals. The baseline food intake was measured the day before treatment and 48 hours post dosing. The animals were dosed with four different doses of liraglutide intravenously in the tail vein under brief isoflurane anesthesia. Blood, for determination of plasma exposure, was taken from the sublingual plexus from conscious animals 3 minutes and 24 hours post dosing. The results demonstrated that intravenous dosing under anesthesia and sublingual blood sampling did not affect food intake in vehicle treated animals. The combination of automatic monitoring of food intake and plasma exposure allowed for an EC50 determination for liraglutide of 44 nM ± 7. In conclusion, we have established a state-of-the-art PK/PD rat model, enabling easy and precise comparison of EC50 values of potential lead candidates without introducing additional stress and interference by the extensive handling of the animals.

P184 Involvement of Corticotropin-Releasing Factor Type 1 Receptor (CRF-1) in Nociceptive Behavioral Responses in Mice

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The CRF-1 receptor has been associated with disorders in processing nociceptive information. Transgenic (knockout) models are often a useful strategy in pain research. We evaluated the role of the CRF-1 receptor in nociceptive responses using wild type (wt) and CRF-1 knockout mice (KO), in a C57BL/6J background. Chemical (acetic acid writhing test, AAWT), thermal (hot plate and tail flick), and mechanical (von Frey) stimuli were used in both genotypes. Moreover, we assessed mechanical thresholds and plasma extravasation (PE) in a mouse model of incisional pain. The *number of writhes* (AAWT) in KO mice (41.25 ± 11.36) was significantly higher compared to wt (23.80 ± 4.71, $P < 0.01$). However, no differences were observed between genotypes in the *hot plate* (10.30 ± 1.4sec and 12.10 ± 2.9sec), *tail flick* (4.60 ± 0.84sec and 4.30 ± 0.48sec) and *von Frey* (0.89 ± 0.18g; 1.10 ± 0.03g) tests. Plantar incision significantly decreased mechanical thresholds on postoperative days 1, 2 and 21. (-76.06 ± 10.53%, -80.42 ± 9.55, and -18.91 ± 16.54%), and KO (-93.65 ± 0.57%, -53.34 ± 13.36% and -34.98 ± 16.33%), with no significant differences between groups. Baseline values for PE were 0.20 ± 0.12UA/g and 0.22 ± 0.08UA/g in wt and KO respectively. On postoperative day 2 PE was significantly increased in KO mice ($P < 0.001$; 1.13 ± 0.05 UA/g and 0.19 ± 0.18 UA/g, in wt and KO respectively). The results show that the CRF-1 receptor is involved in the nociceptive responses to chemical but not thermal or mechanical stimulus, also contributing to the local inflammatory response to tissue injury (incision).
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P185 Modeling of Human Cancer Diseases on Immunodeficiency Mice in Center of Genetic Resources of Laboratory Animals

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Human glioblastoma are the most lethal primary brain tumor with a median survival of patients less than twelve months because of resistance to radiation and other treatments. Glioblastomas are diffuse tumors with invasion into normal brain structures that frequently recur or progress after radiation as focal masses. It's suggested that only a fraction of the tumor cells is responsible for regrowth. For the rapid screening and evaluation of potential therapy of malignant gliomas an animal model is commonly exploited. Human tumors were induced by intracerebral injection of 5 µL U87 line cells ($500^{*}10^{3}$ cell per injection) in 5 females of severe combined immunodeficiency mice (SCID). To examine brain tumors we utilized T1- and T2- weighted magnetic resonance imaging (MRI) techniques. The results demonstrate that the metastatic behavior of the human glioblastoma did not depend on brain structure. In all cases tumor growth were exponential, began not earlier than 3 weeks after injection and were followed by body weight loss, up to 1/3 body mass. We visualized gliomas with contrast enhancement after intravenous administration of manganese using T1-weighted MRI method (Multislice Multi Echo (MSME)). Thus, we have established one more animal model of human glioblastoma tumor and method for it analysis and testing of therapeutic techniques and medications.

P186 Ventricular Tachycardia Inducibility in 2 Swine Models of Healed Myocardial Infarction

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A large animal model of reproducible post-infarct ventricular tachycardia (VT) could be useful in the preclinical evaluation of different therapeutic approaches, as well as for training in electroanatomical mapping and arrhythmia ablation techniques. Our aim was to evaluate and compare the inducibility of post-infarct VT in two swine models of myocardial infarction. For this purpose, an anteroseptal myocardial infarction was induced in 12 Large White pigs (35-40kg) by either 150 minutes balloon occlusion of the LAD immediately distal to the origin of the first diagonal branch ($n = 6$) or by the administration at this same site of 3ml of 100% ethanol through an inflated balloon, while under

general inhalant anesthesia. Cardiac troponine I (TpnI) was measured to monitor the infarction at baseline and at 6 hours after intervention. An electrophysiological study including endo and epicardial left ventricular voltage mapping was obtained 5-6 weeks after model creation and VT induction was attempted. Two animals died during infarction (one from each group). Infarct creation was successful in all surviving animals. No significant differences in TpnI were seen between groups, but a significant ($P = 0.01$) increase was seen 6 hours after infarct, from 0.07 ± 0.1 µg/L to 14.29 ± 11.29 µg/L. Successful endocardial mapping was achieved in all animals. One animal from the occlusion group died from spontaneous untractable ventricular fibrillation before completing the epicardial mapping. VT was successfully induced in all remaining animals (90%). Both experimental infarction techniques can successfully and consistently be used to induce reproducible VT induction associated to the healed scar in pigs.

P187 Surgical Removal of Tonsils in Pig

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The tonsils of the soft palate in swine are secondary lymphoid organs that have great importance in the immune barrier. This tissue is particular characteristic lymphoid in this species and plays an important role in the mechanisms of immunization in the host against most of pathogens that used the mucosal tissue as door entrance. Unlike the rest of lymphoid tissue, the mechanisms of action of these tonsils are mediated mainly by IgG and IgA. The objective of our work was to design a model of pigs in which this particular mechanism of immune function was canceled. This experimental model could be very interesting for the study of issues related to advances in technology for vaccines development. To achieve this goal, we performed a surgical removal of the tonsils in a group of 12 minipig aged between 2 and 4 months. Soft palate tonsils of pig have special characteristics which prevent the extraction following the techniques described for the ablation of the palatine tonsils in other species. For the surgical approach we used a cold light source connected to a column of endoscopy and laparoscopic equipment provided sterile cautery was introduced into the oral cavity to perform the procedure in the area of the soft palate. The use of these instruments allowed the complete removal of the tonsils by perimeter incision avoiding damage any other structures and controlling bleeding, which facilitated a quick recovery of the animals in the postoperative.

P188 Biochemical Parameters in the Minipig Sach Strain: Comparison of 2 Methods of Measurement (Electrolytes and Blood Glucose)

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From 1983 to the present, research models in porcine has gained great interest to the experimental surgery research teams in our institution. In experimental surgical procedures, it is essential to obtain information about biochemical and electrolyte parameters for monitoring and observation of the clinical course of the animals during the surgical sessions and postoperative survival time. The aim of our study was to establish a baseline recording of these parameters as a reference to compare the results obtained in the course of these procedures. For the present study, venous blood samples were collected from minipig Sach strain (36 females and 33 males). Glucose levels, ALT, AST, alkaline phosphatase, LDH, CK, amylase, creatinine, urea, total protein, Na^+, K^+ and total Ca were determined in a modular autoanalyzer cobas c 711. These parameters were measured in three different stages: weaning animals (≤ 10 kg) prepubertal stage (≤ 30 kg) and sexual maturity phase (> 30 kg). Levels of Na^+, K^+ and glucose had a secondary determination using an analyzer Gem Premier3000. The correlation between the values of Na^+, K^+ and glucose obtained in the two methods of measurement was performed. According to literature sources, minipig Sach strain have relatively elevated urea mean values in relation with those reported by other authors for this specie. However, creatinine and Na^+ mean levels were relatively low compared with other minipig strains.

P189 Hemodinamic Parameters in Minipig Sach Strain

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Since 1983 to the present, the porcine model has gained great interest to the experimental surgery research teams at our institution. In experimental surgical procedures, it is essential to obtain information on haemodynamic parameters and homeostatic balance, monitoring and controlling the vital signs of animals in order to validate the experiment performed and results obtained. The aim of our study was to establish a baseline recording of these parameters as a reference to compare the results obtained in the course of these procedures. For the present study, venous blood samples were collected from minipig Sach strain (36 females and 33 males). Levels of pH, PaO_2, $PaCO_2$, Na^+, K^+, Ca^{++}, glucose, lactate, hematocrit, Ca std at pH (7.4), HCO_3^-, HCO_3^-std, CO_2 total, Beecf, Be (B), saturation (SO_2) and THbc were determined in an analyzer Gem Premier3000.These parameters were measured in three different stages: weaning animals (≤ 10 kg.), prepubertal stage (≤ 30 kg) and sexual maturity phase (> 30 kg). Weaning animals showed Na^+, Hct, Total CO_2, Beecf, Be(B) and THbc levels significantly lower than the rest of animals, while lactate levels resulted significantly higher than them. The values obtained for hematocrit and THbc with lactate levels significantly higher than other pigs, could be explained by iron deficiency manifesting swine and causes an inadequate supply of oxygen to the tissues, which increases cellular stress.

P190 Experimental Study of the Mechanical Effects in the Pathogenesis of Amniotic Band Syndrome in the Rabbit

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Amniotic band syndrome (ABS) is a congenital disorder characterized by limb constrictions and its exact pathogenesis is unknown. Though theories have been advanced to explain the condition's origin, none have been scientifically validated. Our purpose is to assess whether annular external compression of a fetal rabbit limb will produce a band of subcutaneous fibrous tissue characteristic of ABS. We operated one limb of 10 different rabbit fetuses, at 21 days of gestation. The extremity was ligated with a suture at the infracondylar level. At 30 days gestation, each fetus was delivered by caesarean section. Two fetuses were found to have been aborted. Limbs were analyzed histologically using different techniques. In the hematoxilin-eosine staining of each limb, we observed narrowing of the epidermis, dermis and hypodermis in the area of constriction. In this zone the sebaceous glands and hair follicles had disappeared. Inspection of the trichromic stains, used to recognize support fibers and differentiate them from muscle, did not demonstrate any new, dense connective tissue of annular distribution in the zone of external compression. The segment distal to the constriction showed subcutaneous edema, with expanded lymphatic vessels. Our results demonstrate compaction of musculoskeletal tissues at level of the constriction, but the ligature failed to produce new dense connective tissue, the clinical and histologic features present in ABS. Annular

external compression of a fetal rabbit limb does not induce development of new fibrous tissue; therefore this experimental study does not support the theory of a mechanical exogenous pathogenesis in ABS.

P191 Characterization of Bone Turnover in a Mouse Model of Senile Osteoporosis

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The senescence accelerated mouse prone 6 (SAMP6) exhibits significant deficits in bone quality as compared to the age-related control strain, the senescence accelerated mouse resistant 1 (SAMR1). Although the low bone mass phenotype in SAMP6 mice is well described, data pertaining to bone turnover is lacking. We used time-lapsed in vivo micro-computed tomography to study age related changes in bone microstructure in both strains over a period of 12 weeks. The 6th caudal vertebra of 10 female SAMP6 and 10 SAMR1 mice was scanned at week 8, 10, 12, 14, 16, 18 and 20. Static as well as dynamic parameters were evaluated. At week 8, SAMR1 demonstrated a 37% (± 1.6%; $P < 0.001$) higher trabecular bone volume fraction (BV/TV) than SAMP6 at the same total volume. BV/TV was decreased by about 15% in both strains at week 20 as compared to week 8 maintaining a significant difference between the two strains ($P < 0.001$). In SAMR1, this loss was attributed to a 17% (± 0.2%; $P < 0.01$) reduction in the number of trabeculae (Tb. N), while in SAMP6, it was shown to be a combination of both reduced Tb. N (-7 ± 0.0%; $P < 0.01$) and additional thinning of the trabecular network (-5 ± 0.1%; $P < 0.001$). Cortical thickness increased by 16 ± 0.0% in both strains over the 12-week period ($P < 0.001$). In SAMP6, bone formation and resorption actually exceeded that of SAMR1 at selected time points ($P < 0.05$). In conclusion, despite us confirming SAMP6 mice as having lower bone mass, we could not demonstrate a reduced bone turnover in these mice in comparison to age-matched SAMR1.

P192 Stem Cell Therapy for Posterior Arch Bone Regeneration in a Fetal Sheep

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Myelomeningocele (MMC) is a congenital malformation originated by a closure defect at the posterior aspect of the spinal column. It causes progressive damage of the unprotected fetal neural tissue through gestational-age advance. Herein we describe the sheep as experimental model for MMC fetal surgery using a cellular-therapy approach. All surgeries were made under general anesthesia (induction with propophol, maintenance with isoflurane). Nine pregnant sheep underwent surgery at 75Th gestational-day for MMC-defect surgical creation. After laparotomy, hysterotomy was performed to expose the fetus. A MMC-like defect was created in the fetus by surgical resection of three lumbar vertebral arches and dura-mater.100ml of Amniotic fluid was collected to isolate stem-cells. Animals recovered from anesthesia and at day 95th underwent a second fetal-surgery, in order to repair MMC. Animals were assigned to 3 groups according treatment: (1) stem-cells imbibed in platelet-poor plasma gel (PPP), (2) stem-cells assembled with Demineralized-Bone-Matrix (DBM) and PPP and (3) PPP scaffold mixed with DBM without cells. At day 150, the lambs were delivered by caesarean-section, euthanized and histologically examined. The application of stem-cells in a PPP-scaffold did not appear to stimulate bone regeneration. When DBM was added within the mixture, de novo bone formation was observed at the covered area. The application of DBM with no presence of stem-cells resulted in reabsorption of the demineralized bone pieces. The Combination of stem-cells derived from amniotic fluid and DBM-based scaffold can be a promising approach for prenatal MMC repair by promoting bone formation in the posterior vertebral arch area.

P193 Improved Monitoring of Orthotopic Brain Tumor Growth by Comparison of In Vivo Fluorescent and Bioluminescent Imaging

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Malignant gliomas are aggressive brain tumors for which no curative treatment is currently available. Researchers heavily rely on appropriate clinically relevant animal models that recapitulate all the characteristics of this devastating disease. We have established orthotopic brain tumors from patient derived glioma biopsies in mice and rats. Here we apply and compare noninvasive bioluminescent and fluorescent in vivo imaging technologies (IVIS, Calipers) to optimise our animal experiments and reliably determine tumor take, tumor progression and estimate tumor volume. We show that for orthotopic brain tumors, fluorescent cell labeling and quantification is more reproducible and more robust compared to luciferase based bioluminescence imaging. With fluorescent in vivo imaging a tumor growth curve can be obtained, which allows to determine the optimal time of drug administration, the speed of tumor growth and the time of animal euthanasia. Using this noninvasive in vivo monitoring technology, animals can be sacrificed before they display neurologic symptom or symptoms of pain. Detailed results will be presented and the utility and applicability of different noninvasive imaging modalities will be discussed.

P194 Aged Rats Show Memory Deficits, Aging Related Changes in Brain Metabolites by 1H-MRS and Pituitary Tumors by MRI

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Cognitive decline is closely related to aging in the human population. The aim of the current study was to evaluate in female Fischer rats memory and learning deficits, levels of synaptic markers, in vivo changes in hippocampal metabolites using magnetic resonance spectroscopy (1H-MRS), and monitoring the overall brain anatomy with emphasis on any aged-related alterations. Learning and memory capacities of 23-month-old aged rats and young 3-month-old control rats were tested in the Morris Water Maze (MWM). In addition, the aged rats and the young control rats were subjected to brain imaging by in vivo MRI and in vivo ^{1}H-MRS. Finally, brain tissue samples were collected and mRNA levels of cortical PSD95 were analyzed by qPCR. In MWM test, significant learning and memory deficits between young and aged rats were observed.1 H-MRS analysis of hippocampal in vivo metabolites showed significant changes associated with the aging process: inositol levels had increased whereas glutamate and N-acetylaspartate levels had decreased. Furthermore, levels of cortical PSD95 mRNA levels were significantly decreased in the aged rats. The most striking discovery was that more than 65% of the aged 23month-old rats showed pituitary tumors. However, the presence of tumors did not correlate with any alteration in functional or cognitive parameters as analyzed in the MWM. Summarizing, the aged Fischer rats offer a natural model to assess the efficacy of novel therapies aimed to treat age-related alterations.

P195 Prenatal Metformin Exposure in a High Fat Diet Model Protects the Metabolic Phenotype of the Offspring

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Auto-cannibalism is a challenging problem in experimental flap surgery especially in rats. Several methods have been described to prevent auto-cannibalism such as drugs, bandages, vests or caging alone. These methods may cause some morbidities including irritation of rat comfort and blocking free motion, rats can easily remove them by licking, scratching and biting. Also caging alone is important sources of stressors, such as anxiety, boredom, fear, loneliness and increasing the cost of studies. In a pilot study; 12 rats weight varies 300-330 grams, separated to 2 experimental groups. After the surgical procedure of flaps, first group dressed in vest as commonly used, in the second group elastic adhesive bandage was applied. In the first group 2 rats opened each others sutures, one rat flap is eaten by other rats. In the first group, feed consumption and movement possibility were decreased. In the second group, bandage did not affect feed consumption and the mobility of rats, none of the bandages were opened and there was no flap loss. In addition, second group post-operative weight loss was observed less than the first group ($P < 0.005$). Depending on the results of pilot study, elastic adhesive bandages are being used in all surgical flap models in our laboratory. Totally 179 rats were used in different flap studies. Elastic adhesive bandages reveal that the flap loss rate is %0. Results show that the elastic adhesive bandage is useful for the experimental flap studies to prevent auto-cannibalism.

P196 Evaluation of Fine Motor Skill Impairment of R6/2 and zQ175 Knockin Mice of Huntington Disease

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Huntington's disease (HD) is an autosomal neurodegenerative disorder, characterized by severe behavioral, cognitive, and motor deficits. Fine motor skills, such as finger-tapping rhythm and rate, are applied for an early diagnosis of HD. Here, we investigated fine motor skill characteristics of R6/2 and zQ175 knock in (KI) Huntington's disease mice. The aim of this study is to characterize phenotypic HD-like fine motor deficits in zQ175 KI and R6/2 mice by using Motorater, a novel automated high precision movement analysis system. R6/2 mice and their wildtype littermates (WT), and zQ175 homozygote and WT mice were characterized. The mice were evaluated in Motorater apparatus, developed to detect fine motor skills in rodents. The performance of the mice is assessed in three different conditions, walking, wading and swimming, and each activity is recorded with high speed camera. Rodents can be imaged and analyzed simultaneously from three spatial dimensions, from beneath and both sides to allow comprehensive kinematic analysis. The movement of different joints and various body parts respective to each other is evaluated and correlations analyzed to reveal disease specific defects. Preliminary data indicates phenotypic HD-like changes in essential fine motor movement components in R6/2 and homozygote zQ175 mice that cannot be detected using more conventional motor tests. This new method is applicable not only to study fine motor defect development in rodent models of HD and other motor impairment diseases, but also may offer a sensitive tool to investigate efficacy of therapeutic approaches in early and subtle motor skill changes.

P197 Early Detection of Cognitive Deficits in Newborn Mice

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Mouse models are increasingly used to investigate genetic contributions to developmental disorders in children. In particular, early cognitive assessment in newborn mice is critical to evaluate pediatric drug efficacy and toxicity. Unfortunately, learning procedures developed in adult rodents are not suitable for testing newborn rodents because of their motor and sensory immaturity. Furthermore, newborns have far greater capacity for learning but less capacity for memorizing. Therefore, developing such tests for newborn mice is a priority challenge for neurogenetics and pharmacological research. Our goal was to develop a conditioning method well suited to high-throughput cognitive screening in newborn mice. To this end, we developed an odor preference conditioning test using ambient temperature as an unconditioned stimulus (US) and artificial odors as conditioned stimuli (CS). First, we showed that mouse pups move toward the thermoneutral temperature when offered a choice between a thermoneutral and cold environment, thus showing thermotaxis. Second, we conducted a classic conditioning paradigm in pups aged six to ten days. In terms of central nervous system development, this period corresponds to extreme prematurity to early postterm period in humans. During acquisition, the pups were alternatively exposed to odor CS paired with either cold or warm temperatures. Immediately after acquisition, the pups underwent a two-odor choice test, which showed preference for the odor previously paired with the warm temperature, thus showing conditioning. The proposed paradigm is easy to conduct, and requires modest experimenter interference. The method is well suited for high-throughput screening of early associative disorders in newborn mice.

P198 Intermittent Negative Pressure Ventilation to Ressuscitate One-Day-Old Newborn Mice

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Phenotyping of genetic animal models in the early neonatal period has become an important step toward understanding the molecular basis of human diseases. Unfortunately, early postnatal lethality is common in a large number of genetically engeneered mice, thus precluding any possibility of performing in vivo analyses and validating the model. Providing ventilatory assistance to newborn mice may help them to overcome the critical period of early postnatal respiratory failure and make these mouse models suitable for in vivo phenotyping studies. We report here a new prototype of negative pressure ventilator able to deliver small tidal volume at a high respiratory rate, close-fitted to newborn mice ventilatory pattern. We tested the efficiency of this new ventilator to ressuscitate newborn mice after cardiorespiratory arrest artificially induced the day of birth (P0) or the day after birth (P1) by forced inhalation of 10% isoflurane. Survival rate in the reanimated (n = 50) and nonreanimated (n = 48) groups were respectively 90 % and 25 %, showing that our negative pressure ventilator is effective in our model of neonatal cardiorespiratory arrest. We also investigated the consequences of this ventilatory ressuscitation on normal physiologic development at P2 and P7. No difference was found between the animals of the control and reanimated groups, showing that ventilation by negative pressure did not induce severe pulmonary sequellae.

P199 Animal Models for Pediatrics: Novel Methods for Phenotyping Newborn Rodent Pups

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Newborn mice and rats are a suitable model for pediatric research: they resemble preterm human infants regarding neurodevelopmental characteristics and mutant models of pediatric diseases are available at an increasing proportion. To study these animals early in their life is essential i) in case of early death of the model, ii) to avoid the effect of functional recovery, iii) to understand the ontogeny of the pathology at early stages of development and to comply with EMEA guidelines on preclinical testing in juvenile animals for pediatric drug development, However, phenotyping of newborn mice faces major difficulties due to the small size of the pups and their sensory and motor immaturity. To overcome the lack of dedicated equipment, we developed noninvasive devices suitable for high throughput screening: PhysioPups (patented device) assesses vital functions: measurement of respiratory patterns, electrocardiogram (ECG), body temperature, gross body movements, and ultrasonic vocalizations. Four animals can be tested simultaneously under controlled conditions of temperature and gas composition. NeoGAIT assesses sensorimotor development: it detects the contact patterns of the animal with the floor (infrared technology) that are indicative of the gait development. MemoryPups assesses memory and associative learning abilities. The methods are based on olfaction and thermotactile sensitivity, the only sensory functions operating at birth. This platform has no equivalent worldwide. It is currently used for the characterization of genetically engineered mice, including models of genetic diseases (autism, Prader-Willi, Ondine's syndrome) and for toxicity studies of anti-infectious drugs used in neonates (eg. European TINN project).

P200 The Effect of Anti-EGFR Therapy in Combination with Anti-Ganglioside Treatment in Spontaneous Lung Metastasis Models in C57BL/6 and BALB/c Transplanted with 3LL-D122 Lung Carcinoma and 4t1 Metastatic Breast Cancer

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Since the beginning of the last century until today, the murine models contributed to the understanding of the pathogenesis of many diseases and the development of new therapies. Lung metastases are as a result of different tumor types and are the leading cause of death from cancer. Is frequently used experimental lung metastasis model to determine the effect of anti-metastatic drugs. However, the spontaneous lung metastasis model more closely resembles the clinical scenario. We studied the relevance of spontaneous lung metastases in C57BL/6 and BALB/c transplanted with two carcinoma cell lines: 3LL-D122 (Lewis lung carcinoma) and 4T1 (metastatic breast cancer) and the treatment with anti-EGFR treatment in combination with anti-ganglioside therapy. First we explored the effect of anti-EGFR therapy (7A7 mAb) in combination with anti-ganglioside treatment (vac. NGcGM3/COMPARED WITHSP) on survival in the models of lung metastases having as endpoint experimentation provided by our CIM-IACC (Institutional Committee for the Care and Use of Laboratory Animals). Second, we evaluated the anti- metastatic effect, proliferation, apoptotic and angiogenic process by histopathological evaluation. The metastasis dissemination involves different stages and the combination therapy was evaluated in three steps in this process: proliferation, angiogenesis and adhesion process. The combination therapy increased survival, impaired pulmonary micrometastases and reduced the angiogenesis process. The complexity of the biology of a tumor should be explored in two or more mouse models as it provides answers for reliable and reproducible biologic significance.

P201 CD38 Expression as a Differentiating Token of Age-Related and Amyloid-Induced Changes in the Limbic System of the Brain

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We suggested that significant modification in the brain after exposition of enriched environment (EE) may be partly regulated by neuronal and/or astrocytic CD38. The main objective is to study processes of brain plasticity in normal and pathologic development which associated with impairment in social and nonsocial behavior. Exposition of EE was for 60 day. The animal model of neurodegeneration induced by intracerebral injections of ß-amyloid into the CA1 hippocampal region of rats bilaterally. Control rats of similar age (9 month) will be identically injected with the phosphate saline buffer. Old-aged mice will be used as a group of physiologic ageing (19-21 month). At two week postinjection brains were removed. We have found increased anxiety, reduced cognitive function, social interest and social memory in rats with experimental model of Alzheimer disease (AD). EE stimulates cognitive function in 9 month animals. EE has no influence on the cognitive function of the damaged brain or aging brain (19-21 months.) however, EE reduces anxiety and increases search activity in rat model of Alzheimer disease. Enriched environment provides improved adapt to the new environment, also EE has positive impact on the social memory and social interest in older animals and animals with AD. Rats with experimental AD have shown reduced expression of CD38 in hippocampus and cortex, but not the amygdala. We first show that the intensity of CD38expression in the amygdala may be a token that allows differentiating of age-related and amyloid-induced changes in the limbic system of the brain.

P202 The Closed-Chest Porcine Model of Reperfused Myocardial Infarction: Comparison between Different Occlusion Regimes

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The aim of this study was to evaluate the appropriate location and duration of left anterior descending coronary artery (LAD) occlusion to create an optimal porcine acute myocardial infarction (AMI) model. Through a percutaneous femoral approach a balloon catheter of proper size was advanced into the LAD of 30 Large-White pigs. Balloon inflation was performed proximal (45-min; $n = 10$) or distal (90-min; $n = 10$) to the origin of the first diagonal branch. In a further 10 swine balloon was inflated below the second branch (150-min). Troponin I (TpnI) was measured before and 24 h postocclusion. Delayed enhancement images were acquired by magnetic resonance (MR) at baseline and 1month post-infarction. Procedural incidence of ventricular fibrillation (VF) and mortality were compared among the three groups. At 24 h, TpnI increased from 0.02 ± 0.01 µg/L (baseline) to 4.85 ± 5.96 µg/L (45-min group) and ≥25.00 ± 0.00 µg/L (remaining groups). MR revealed large infarcts in all subjects belonging to the 90 and 150-min regimes, while necrosis was absent in the 45-min occlusion animals. Four animals died during infarction and a further 5 required 1 to 56 defibrillator shocks (45-min group).70% of the animals that underwent 90-min occlusion and 80% of the pigs submitted to 150-min occlusion suffered from VF, requiring 1 to 6 and 1 to 10 defibrillator shocks, respectively. LAD occlusion was safer when a distal balloon location was used. Although no significant differences were seen between 90 and 150-min occlusions, a shorter occlusion translates into lower anesthetic and staff costs, whereby in our opinion a 90-min regime appears to be the most appropriate AMI model.

P203 Intracoronary Administration of Porcine Cardiac Stem Cells in a Swine Model of Acute Myocardial Infarction: Evaluation of Safety and Efficacy

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The aim of this study was to evaluate in a swine model of acute

myocardial infarction (AMI) the safety and efficacy of an intracoronary delivered 25x10^6 porcine cardiac stem cell (pCSC) dose. Through a percutaneous femoral approach an AMI was induced in 10 Large-White pigs by a 90-min balloon occlusion of the left anterior descending coronary artery immediately distal to the origin of the first diagonal branch. Intracoronary administration was carried out 7 d post-AMI injecting either 25 × 10^6 pCSCs (n = 5) or placebo (n = 5). Troponin I (TpnI) levels were measured before and 24 h postinfarction, as well as before and 24 h postinjection. Ejection fraction (EF), end diastolic (EDV) and systolic volumes (ESV) were evaluated by magnetic resonance (MR) before and 10w after intracoronary injection. After euthanasia TTC staining of the sliced hearts was performed. Successful infarction was demonstrated by a significant increase in TpnI from baseline (0.041 ± 0.044 µg/L) to 24 h post-AMI (25.000 ± 0.000 µg/L); however, no significant elevation was evident 24 h postinjection. At 10w EF was higher in the cell-treated than in the placebo group (51.48 ± 5.05% compared with 41.72 ± 10.86%; P = 0.190) while EDV and ESV were lower (85.09 ± 11.07 mL compared with 106.47 ± 17.42 mL; P = 0.063 and 41.69 ± 9.43 mL compared with 62.75 ± 19.31 mL; P = 0.111). TTC staining revealed large infarcts in all placebo animals, necrotic lesions in the pCSCs group were smaller and more diffuse. Intracoronary administration of a 25 × 10^6 pCSCs dose appeared to be safe as demonstrated by TpnI. Although differences between both groups were not statistically significant, there was a trend towards improvement of cardiac function in the cell-administration group.

P204 Cardiac Biomarkers Validation Study in a Porcine Acute Myocardial Infarction Model

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In the last decade, there has been a substantial increase in the number and effectiveness of laboratory biomarkers for the assessment of cardiac damage. Most of these biomarkers are measured by immunoassay techniques, the cross reactivity with human samples in animal tests is difficult to predict and often varies according to the tested species. Therefore the validation of these markers, based on the species studied, results essential to verify the accuracy of the provided results. The study was carried out using blood from one Large White pig subjected to an endovascular acute myocardial infarction model (AMIM). The validation of the following parameters was performed using an immunoassay analyzer: Troponin I, CKMB, and Myoglobin; the measurements were performed at two different times: before the AMIM (baseline) and 24 hours after the infarction. Coefficients of variation below 4% were obtained in all the parameters studied at the baseline sample; all of them were in the normal clinical range for the studied enzymes. In the 24 hours after infarction samples, an increase in all the studied enzymes were obtained, that increased was strongly significant in the case of Troponin; obtaining coefficient of variation values below 5% in the repeatability study and below 3% in the intermediate precision study. The obtained results are consistent with previous studies in which a high increase in the release of troponin is observed until 12 to 24 h after infarction. The coefficients of variation obtained are optimal, and because of this the methods used can be considered precise and repeatable.

P205 Red Blood Cell Parameters in Minipig Sach Strain: Comparison of 3 Methods for Measurement

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From 1983 to the present, the interest of experimental of surgery in porcine has been increasing by many research teams at our institution. Before any surgical procedure, it is essential to obtain information on red cell parameters to ensure the quality of the experimental procedure and to validate the results obtained in the experiment performed. The aim of our study was to measure the red cell parameters in swine blood samples with a human sample analyzer to test if the results obtained could be extrapolated to porcine. For the present study, venous blood samples were collected from minipig Sach strain (36 females and 33 males). RBC, hemoglobin, hematocrit, MCV, MCH, MCHC, RDW, platelet count, mean levels were determined in an auto-hematology analyzer BC-2800 Vet previously calibrated for swine. All mean levels for these parameters were secondary determined in an analyzer Coulter LH-750. In addition, hemoglobin and hematocrit levels were measured in an analyzer Gem Premier3000. These parameters were measured in three different stages: weaning animals (≤ 10 kg), prepubertal stage (≤ 30 kg) and sexual maturity phase (> 30 kg). Correlations between the values obtained from the three methods and for all possible parameters for each method were performed. Significant differences appeared in many of the red cell parameters according to stage groups, standing out the importance of specifying the characterization of these standard values for each swine strain.

P206 Animal Model of Chronic Anemia in Mice: Lung Expression of DMT1, Ferroportin, Ferritin and Hemochromatosis Protein

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Anemia of chronic disease is a multifactorial pathology characterized by an inappropriate regulation of erythropoiesis and iron metabolism. We studied iron cycle in a mice model of chronic anemia, studying the localization of key proteins as Ferroportin (FPN), divalent metal transporter 1 (DMT1), Ferritin and the hemochromatosis protein (HFE) in lung cells. CF1 female mice (n = 6/group, paired design) were grouped into: Group 1) Chronic Anemia: bleeding every 3 days for 32 days; Group 2) Without chronic anemia. We followed the international rules of use of laboratory animals. Chronic anemia: Hb levels 10.3 g/dL ± 0.3. Without anemia: Hb levels 15.2 g/dL ± 0.5 (P < 0.05). Immunolocalization of iron proteins using specific antibodies: a) chronic anemia: 1) DMT1 and FPN: apical in bronchial cells and cytoplasmatic in alveolar cells and macrophages; 2) HFE: apical in bronchial cells; 3) Ferritin: cytoplasmatic in macrophages and bronchial cells; B) without chronic anemia: 1) FPN, DMT1, HFE: cytoplasmatic in bronchial-alveolar epithelium and in macrophages; 2) Ferritin: cytoplasmatic in macrophages and in bronchial cells. The chronic anemia model showed changes in the distribution of iron cycle proteins associated to iron importation (DMT1), exportation (FPN), regulation (HFE) and storage (Ferritin) in lung tissue. A novel contribution of our study was to show that lungs play regulatory functions in iron physiology in erythron and iron metabolism diseases, similar to that described in classic iron associated tissues.

P207 Maternal Undernutrition during Pregnancy and Accelerated Postnatal Growth Animal Models Are Associated with Early Fat Accumulation in Male and Female Pups

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Cardiometabolic syndrome (CMS) is one of the leading causes of morbidity and mortality worldwide. Epidemiologic studies demonstrate that maternal undernutrition during pregnancy and postnatal accelerated growth, are risk factors for CMS development in adulthood. We aimed to assess the validity of animal models for the study of CMS risk factors at early stages of postnatal development. At weaning, we compared the pups of SD rats fed ad libitum during gestation, standardized to 12 pups/litter (Control, C), with those of rats fed 50% of the C rats daily intake during the second half of gestation, standardized to 12 pups/litter (Restricted, R) and with pups of rats fed ad libitum during gestation and reduced to 4 pups/litter (Accelerated Growth, AG). We determined: tibia length, body weight and brown, mesenteric, peri-

gonadal, peri-renal and subcutaneous adipose tissue weight indexes (relative to body weight). Compared to C pups, both male and female R offspring exhibited significantly smaller body weight and tibia length but significantly higher peri-renal fat index. In AG male and female pups we observed a significant increased body weight, tibia length and peri-renal fat index, while subcutaneous fat was increased only in males. Despite the poorer body growth produced by undernutrition during pregnancy it produces peri-renal fat accumulation. Accelerated growth does not alter postnatal growth but also associates with peri-renal fat accumulation. Since peri-organic fat is a risk factor for CMS, we suggest that the proposed animal models are useful in research of fetal and perinatal programming of cardio-metabolic diseases and sex-dependent effects.

P208 Radiopaque Thrombus Preparation for Endovascular Mechanical Thrombectomy Devices Test in a Preclinical Animal Model of Acute Stroke

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The goal of stroke treatment is the fast recanalization of the occluded arteries to restore cerebral blood flow. A new treatment approach is the endovascular mechanical thrombectomy, with potential of accelerated revascularization and improved effectiveness in disposing large clot burdens. The development of animal models of site-specific thromboembolism, to test emerging techniques for endovascular therapy, is necessary. Radiopaque thrombus are an important tool assessing thrombectomy device performance. The current models use radiopaque thrombus barium-composed, with different mechanical properties from the ones found in the clinical settings. Herein we detail the preparation of a new radiopaque thrombus with improved mechanical properties, describing ex vivo and in vivo behavior to thrombectomy. Venous pig blood was mixed with several concentrations of different radiopac contrast agents (iopamidol, iomeprol, meglumine-amidotrizoate, barium-sulfate). Several concentrations of thrombin (2.5 to 120 IU/mL) and fibrinogen (0.1 to 7.5 mg/mL) were added. Thrombectomy was performed ex vivo in a simulation model to select the best thrombus for posterior in vivo study using 3 pigs. Under general anesthesia, percutaneous femoral arterial catheterization was used to thrombus delivery in carotid artery and to perform the endovascular thrombectomy. Thrombus-device interaction, dislocation or fragmentation was recorded. Iomeprol-composed thrombus with highest concentration of thrombin and fibrinogen presented the best behavior to thrombectomy. In the in vivo study the thrombus could be delivered to the targeted vessel without fragmentation or dislocation, presented radiopacity, and good interaction with thrombectomy device. The developed radiopaque thrombus is an effective tool to accurately test thrombectomy devices with potential future application in the stroke treatment.

P209 Effect of Chios Mastic Gum Administration on Lipid and Glucose Metabolism in Streptozotocin-Induced Diabetic Mice

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Chios mastic gum, a resin produced from *Pistacia lentiscus var.Chia*, may exert beneficial properties for cardiovascular health. This study investigated the impact of mastic gum administration on lipid and carbohydrate metabolism in streptozotocin-induced diabetic mice. A total of 27 diabetic 12-week-old male C57bl/6 mice were separated into 3 groups: NC (n = 9) control; LdM (n = 9) animals received low dose mastic (20 mg/Kg body weight); HdM (n = 9) animals high dose mastic (500 mg/Kg body weight). All mice became diabetic with intraperitoneal injection of streptozotocin (40 mg/Kg body weight). Serum lipid and glucose levels were determined at baseline, 4 and 8 weeks after the induction of diabetes. Serum adiponectin and resistin levels were also measured after euthanasia, while hepatic lesions were evaluated by haematoxylin-eosin staining.4-week period mastic gum administration resulted in decreased serum glucose and triglyceride levels in both LdM and HdM groups whereas body weight levels were reduced in LdM group compared with controls. At the end of the experiment, LdM group presented decreased serum glucose, total cholesterol, LDL-cholesterol and triglyceride levels, decreased body weight and improved HDL-cholesterol levels compared with the control group. HdM group had ameliorated serum triglyceride levels. Hepatic steatosis observed in the control group was partially reversed at the LdM and HdM groups. Chios mastic gum administered in low dosages improved disturbed glucose and lipid levels in experimentally induced-diabetic mice, while alleviated hepatic damage. Further clinical studies are required to investigate the effects of this natural agent in the prevention and treatment of diabetes and diabetes-related lipid disorders.

P210 Lipopolysaccharide-Induced Cytokine Release Model in Nonhuman Primates

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The use of nonhuman primates (NHP) plays a critical role in drug development, because of scientific and regulatory reasons. However, significant efforts are dedicated to further reduce and optimize the use of animals, by applying highly sensitive ex vivo assays or developing nonterminal mechanistic models. Here we describe a Lipopolysaccharide-induced (LPS) cytokine release model in cynomolgus monkeys and present how determined therapies can ameliorate the inflammatory response. This LPS infusion is also applied in human volunteers and it attempts to mimic some mechanisms of systemic inflammation in human. Initially, different conditions such as LPS type or doses were evaluated in vitro. Posteriorly, the strain 055:B5 from *E. coli* was selected for follow up studies at a dose of 1µg/kg IV (bolus). Applying those conditions in vivo, the four animals initially tested did not show any clinical sign or relevant physiologic alteration in response to LPS (for example, blood pressure, fever). However, it resulted in significant increase of key cytokines (for example, TNF-α, IL-6, IFN-γ), pro-inflammatory molecules (for example, protein C-reactive) or characteristic hematological changes, and importantly, the release of those molecules could be reverted by using determined therapeutic molecules. The value of such mechanistic model is that knowledge about mode of action from selected compounds can be gained within a single experiment (4 animals/group), as several readouts can be monitored. Due to the low discomfort induced, animals can be re-used after a wash out period allowing crossover studies, using the same animal as its own control, and therefore, reduce the inter-animal variability.

P211 The Effect of Biologic Age on the Metabolic Responsiveness of Mice Fed a High Fat Diet

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Mice are widely used in studies investigating the effect of diet on metabolic risk factors such as lipidemic and glycemic profiles. In such studies an important factor that is usually not taken into account is the biologic age of the experimental models. The up-to-date identified experimental confounders do not cover all the parameters that may affect the results of animal studies. The aim of this study was to investigate the effects of a high-fat diet on the metabolic profile, hepatic and renal function in mice of differing ages. For this purpose 2 groups of male C57BL/6J mice were used, consisting of 10 week-old mice and 54 week-old mice. Both groups followed identical high-fat diets for 12 weeks. Younger mice had a smaller increase in body weight, serum total cholesterol, glucose and urea levels compared with older mice. Young mice also had a higher increase in HDL-cholesterol levels than old mice. Our results indicate a necessity for considering the experimental animal's age as a confounding factor when researching or interpreting metabolic studies. Age-adjustment is warranted in all animal research while a uniform approach regarding the age of the animal models should be used in experimental studies. In the future, it is imperative to identify the changes that occur in naturally aging mice in order to ensure more tightly controlled and reliable animal experiments.

P212 Humanization of the Pig. Evolution of an Animal Model for Heart Valve Disease to Mimic the Human Anatomy: From Open Surgery to Percutaneous Intervention

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Mitral valve regurgitation and functional tricuspid regurgitation (occurring secondarily to left-sided heart disease are the most common valvular heart disease in the western world. Surgical option is the treatment of choice, but new devices are always under evaluation. Nowadays percutaneous procedures may be an attractive alternative to surgery for patients deemed to be high-risk surgical candidates. The purpose of this study is to portray the evolution of an animal model to treat these heart valve diseases mimicking human anatomy in order to perform acute and chronic study. We describe our single center experience on over than 500 consecutive procedures on swine pigs, explaining in detail our protocol of surgical technique and anesthesia, from operatory room to intensive care and follow up. We started with operations on full sternotomy, establishing extracorporeal circulation with standard cannulas. The procedures were performed on cardioplegic arrest and controlled with transthoracic or transesophageal echocardiography. We then prosecute with normal or mini left and right thoracotomy. Finally we use tiny accesses setting up extracorporeal circulation totally percutaneous (femoral access) without any mobility impairment. Last frontier is to carry out mitral and tricuspid correction procedures totally percutaneously on beating heart, guided by intracardiac echocardiography. In particular we developed a technique to resemble the transeptal puncture usually performed in percutaneous procedures, creating a newly anastomosed inferior vena cava. We demonstrate that our a valid model to evaluate the feasibility of implantation of innovative cardiac devices and to train the physicians over these novel procedure.

P213 Reduction of Mice in New Assays for Antitubercular Drug Development

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Murine models of tuberculosis are essential tools in drug discovery. The most widely used are models based in the chronic phase of *M. tuberculosis* infection. Although these models reflect reasonably well the efficacy of the major antituberculars drugs, they are very long experiments (months) that needs a large amount of compound (grams) and mice. In order to address the issues of the chronic model, a new short term in vivo assay in mice infected by nonsurgical intra-tracheal instillation has been developed and standardized at GSK. Mice are infected with 10^5 CFU (day 0) and treated with drugs from day 1 (or day 5) to day 8 of the assay. This acute assay efficiently solves the practical caveats of the chronic assay reducing the time from infection to reporting data to one month, requiring about 10% of the product amount needed for a chronic assay and reducing dramatically the number of mice. This work has been part of a FP7 project (ORCHID) and describes the validation of the GSK acute assay for drug discovery. To address this, the in vivo dose-response of a set of standard and new antituberculars was tested in the GSK short term assay and a two-month treatment of mice in chronic phase of lung infection. Our data support that there is a good qualitative correlation between both assays. Overall, our data indicate that the GSK acute assay is as robust as the chronic model to detect antitubercular compounds while using a lower number of mice in shorter assays.

P214 A Murine Model of Plasmodium Falciparum as Potential Model for Transmission-Blocking Studies

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Malaria is one of the most serious public health problems in the world. The *Plasmodium spp* causing malaria are transmitted from humans to mosquitoes by gametocytes, which are the parasite sexual-stage. New drugs to interrupt transmission are essential for eradication of malaria. Currently, there is not a practical in vivo model for *P. falciparum* transmission. We have developed a humanized murine model of P. falciparum blood stages because this parasite is a human-specific pathogen. Nonmyelodepleted NOD scid IL-2Rγ null (NSG) mice are engrafted with human erythrocytes. Engraftment is performed by daily intraperitoneal injection of suspensions of human erythrocytes at 50 % hematocrit. When engraftment reaches 50 % in peripheral blood, mice are infected with *P. falciparum*. The mice are inoculated by i. v. route with 20×10^6-infected erythrocytes. Infection is monitored by flow cytometry every two or three days by sampling 2 µL of peripheral blood. In the present study we test the capability of this murine model to support the generation of gametocytes in vivo. A new strain of *P. falciparum* NF54, the best producer of gametocytes in vitro, able to grow in humanized mice has been developed. Gametocytes are first detected two weeks after infection and are present in peripheral blood at least up to 1 month. Our results suggest that the nonmyelodepleted humanized murine model of *P. falciparum* may be a relevant tool to study factors involved in gametocyte differentiation and a potential model for in vivo transmission blocking studies.

P215 A New Flap Protection Tecniquie for Rats to Prevent Autocannibalism

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Autocannibalism is a challenging problem in experimental flap surgery especially in rats. Several methods have been described to prevent auto-cannibalism such as drugs, bandages, vests or caging alone. These methods may cause some morbidities including irritation of rat comfort and blocking free motion, rats can easily remove them by licking, scratching and biting. Also caging alone is important sources of stressors, such as anxiety, boredom, fear, loneliness and increasing the cost of studies. In a pilot study; 12 rats weight varies 300-330 grams, separated to 2 experimental groups. After the surgical procedure of flaps, first group dressed in vest as commonly used, in the second group elastic

adhesive bandage was applied. In the first group 2 rats opened each others sutures, one rat flap is eaten by other rats. In the first group, feed consumption and movement possibility were decreased. In the second group, bandage did not affect feed consumption and the mobility of rats, none of the bandages were opened and there was no flap loss. In addition, second group post-operative weight loss was observed less than the first group ($P < 0.005$). Depending on the results of pilot study, elastic adhesive bandages are being used in all surgical flap models in our laboratory. Totally 179 rats were used in different flap studies. Elastic adhesive bandages reveal that the flap loss rate is %0. Results show that the elastic adhesive bandage is useful for the experimental flap studies to prevent auto-cannibalism.

P216 Effect of Plant Extracts in Experimental Models of Inflammation in Mice

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Inflammation contributes to acute and chronic human diseases. The aim of this study was to assess the anti-inflammatory activity of plant extracts (*M. officinalis* and *R. officinalis*) on carrageenan-induced paw edema, a model of acute inflammation, and in adjuvant arthritis, a model of chronic inflammation. All the experiments were performed on male adult Swiss mice (weighting 25 to 30 g). Mice were injected with carrageenan into the paw to produce acute inflammation.45 mice were randomly divided in 3 groups. Group 1 (control) did not receive treatment. Plant extracts were oral administered 10 days prior carrageenan in groups 2 (*M.officinalis*) and 3 (*R.officinalis*). Paw volume was measured, pre and post treatment of carrageenan at 0, 1, 4, 24 and 48 h. Percentage of inflammation inhibition induced by each was calculated with respect to its vehicle treated control group. Experimental arthritis was induced according to Freud's adjuvant method. Next, 30 mice were randomly divided into 3 groups. Paw volume was measured, before adjuvant injection (day 0) and post injection, discontinued from days 2 to 40 days. At the end of the experiments, animals were euthanized, and both paws were collected for histopathology and immuno-histochemical profile of TNF-α. Control animals treated with vehicle showed the highest percentage of edema and the lowest percentage of inhibition, indicating therefore greater inflammatory response. Histopathology showed the highest percentage of degree of inflammation on control group. In conclusion, the present study clearly shows a marked anti-inflammatory action due to tested agents in different inflammation models.

P217 Unravelling Noncanonical Functions of Suicide Genes in the Heart in ENDO G and Executioner Caspase KO Mice Models

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Careful design and analysis of experimental animal models to test the relevance of in vitro results is a key to identify therapeutic targets. Despite their role in cell death, apoptotic genes are being involved in nonapoptotic functions. Our work in cultured cells and isolated tissues suggested that the apoptotic nuclease EndoG and the cell death executors Caspase-3 and 7 have nonapoptotic functions in the cardiac muscle. However, previous gross analysis of the respective knock out (KO) mice failed to identify any cardiac phenotype for EndoG deficiency and found embryonic lethality for the global deficiency of executioner caspases. We designed two approaches to further investigate the role of these genes in the myocardium. EndoG KO mouse characterization included morphologic and functional assessment ECG, telemetric monitoring of blood pressure (BP) in 6, 12 and 24-month old animals (n = 5 animals/gender/age/genotype), because EndoG abundance increases with age. An Nkx2.5Cre-Iox conditional KO mouse assured truncation of the caspase genes only in myocytes in the embryonic heart, because caspases are ubiquitously expressed and are abundant in the myocardium only until birth. EndoG-deficient myocytes were bigger than controls and increasing heart malfunction was found in old animals without affecting BP. Septal myocytes in executioner caspase KO young animals were bigger than in controls and this induced ventricular hypertrophy in the adult (n = 5 animals/gender/age/genotype). Thus, the new approaches identified EndoG as a BP-independent regulator of cardiomyocyte growth affecting cardiac function in aged mice and showed that executioner caspases regulate septal myocyte size mimicking septal asymmetric hypertrophy.

P218 Experimental Model of UV Radiation-Induced Cutaneous Photocarcinogenesis in SKH-1 Mice

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Exposition of the skin with solar ultraviolet radiation (UV) is the main cause of skin cancer development. The consistently increasing incidences of melanocytic and nonmelanocytic skin tumors are believed to be at least in part associated with recreational sun exposure. In animal models is well known that UVB is more effective to induce skin cancer than UVA. In the present experiment, 20 SKH-1 female mice were exposed to UV (98.6% UVA/ 1.4% UVB) radiation three times a week for 60 min (80 sessions) on a distance from lamp to dorsal skin of 20 cm. The energy output of the lamps was measured with a UV radiometer. Absorbed energy per session was 21.1 J/cm^{2,} bringing the total energy absorbed by each animal to 1,688 J/cm^2. At the end of the experiments, animals were euthanized, and skin areas exposed to radiation were collected for histopathology. Proliferating cell nuclear antigen (PCNA) was assessed in skin sections as a marker of cell proliferation. All the UV radiation exposed individuals developed malignant neoplasias (skin carcinomas) as well as typical signs of photoaging. PCNA positive cells were observed in suprabasal areas of the epidermis. Given the high similarity between the lesions found in the treated animals and those appearing in human skin following extensive exposure to UV radiation, we consider this model suitable for the study of photoaging and photocarcinogenesis and also for the assessment of antioxidants and photoprotectors effectiveness.

P219 Effect of Spicy Nuggets Spicy Chicken and Aqueous Extract of Red Chili Pepper (*Capsicum frutecens*) at 2 Doses on Gastric Ulcer in Rats

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Several studies indirectly explain that capsaicin found in red chili pepper (RCP) in *Capsicum frutescens* could have an anti-ulcer protective effect on stomach infected with H. pylori by affecting the chemicals the stomach secretes in response to infection. Hence, it was planned to investigate the effect of aqueous extracts of RCP and some spicy foods on the healing of acute gastric ulcer induced by aspirin in rats. Sixty six adult male albino rats (170 g average body weight) of Sprague–Dawley Strain obtained from animal house of the Faculty of Medicine, Um Al-Qura University were classified into 6 groups; one group was used as control -ve while others were given aspirin orally. One group served as control +ve and others administrated with aqueous extracts of spicy nuggets and chicken containing higher concentration of red peppers for

seven days. Length of gastric ulcer, volume of gastric juice, pH, and histopathological changes were examined. Oral administration of aspirin induced gastric ulcer; and mean length of gastric ulcer in control +ve group was higher compared to control -ve group. Oral administration of RCP extract at a higher dose caused much decrease in the length of gastric ulcer. Results showed that the oral administration with all tested chili pepper reduced the length of gastric ulcer, volume of gastric juice, and histopathological changes. Furthermore, the RCP extracts showed increase in pH value of gastric juice. The present study, hence, explains the curative role of RCP for acute gastric ulcer.

P220 Publication Bias in Laboratory Animal Research: A Survey on Magnitude, Drivers, Consequences and Potential Solutions

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Publication bias jeopardizes evidence-based medicine, mainly through biased literature syntheses. Publication bias may also affect laboratory animal research, but evidence is scarce. In an internet survey among laboratory animal researchers in the Netherlands, we assessed laboratory animal researchers' opinions on the magnitude, drivers, consequences and potential solutions for publication bias. We explored the impact of size of the animals used, seniority of the respondent, working in a for-profit organization and type of research (fundamental, pre-clinical, or both) on those opinions. We captured median (interquartile ranges) strengths of beliefs on 5 and 10-point scales (1: totally unimportant to 5 or 10: extremely important).454 researchers responded and considered publication bias a problem in animal research (7 (5 to 8)) and thought that about 50% (32 to 70) of animal experiments are published. Lack of statistical significance (4 (4 to 5)), technical problems (4 (3 to 4)), supervisors (4 (3 to 5)) and peer reviewers (4 (3 to 5)) were considered important reasons for nonpublication (all on 5-point scales). Respondents thought that mandatory publication of study protocols and results, or the reasons why no results were obtained, may increase scientific progress but expected increased bureaucracy. We conclude that nonpublication of ''negative'' results appears to be prevalent in laboratory animal research. If statistical significance is indeed a main driver of publication, the literature on animal experimentation will be biased. This will impede the performance of valid literature syntheses. Effective, yet efficient systems should be explored to counteract selective reporting of laboratory animal research.

P221 Best Practices for Performing Experimental Surgery in Swine

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Swine are one of the primary translational research models and many of the models require anesthesia and surgery as part of the protocol. For over three decades our laboratory has performed thousands of survival surgical procedures in both miniature and domestic swine. These procedures include creation of heart failure models, tissue and organ transplantation, interventional radiology procedures, endoscopic/laparoscopic procedures, device and graft implantations, fetal surgery, wound healing models, and heart valve replacement. Animals should be stabilized for seven days after shipping and socialized prior to performing survival surgical procedures. Specialized caging with enrichment is utilized to acclimate the pigs to the research facility. The design of anesthetic and analgesic protocols specific for the various procedures requires an in depth knowledge of the physiologic effects of the various agents; as well as species specific requirements for perioperative care. In many cases the inappropriate selection of anesthetic protocols may result in severe complications to the outcome of the research protocol, such as those using tiletamine/zolazepam combinations in cardiovascular research. Preemptive analgesia with NSAIDS, opioids and local anesthetics has been demonstrated to reduce the postoperative recovery. Selection of suture and catheter materials which are not inflammatory in swine as well as strict aseptic technique is essential to prevention of postoperative complications. Our laboratory has a continuous <1% complication rate for performing complex procedures in swine using the methods which will be discussed.

P222 Characterization of a Refinement of the "Pylorus Ligation" Model of Rat Gastric Ulceration Resulting in "No Pain" and a More Specific Pharmacological Response

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The pharmacological assessment of the factors for gastric protection of a test substance should involve experimental models that can determine the involvement of cytoprotective factors, as well as their influence on the secretion of hydrochloric acid. The original protocol of pylorus ligation in rats proposed by Shay et al. in 1945, still in use today, provides a latency time of 240 min without considering the effect of postoperative pain in the mechanisms of peptic ulcer. The present work proposes a modification of this experimental protocol by eliminating the pain throughout the postoperative period, as a refinement of the test with consequent improvement of the pharmacological response. Adult male Wistar/Uni rats underwent surgical ligation of the pylorus and were kept anesthetized throughout the experimental period (4 h) in contrast to the other experimental groups that followed the original protocol proposed by Shay et al., 1945. We were able to determine effective doses for a positive control, as well as of a variety of secretagogues in the new experimental protocol proposed. The suppression of postsurgical pain, through the use of anesthesia throughout the experimental period, brought several benefits for the study of gastric acid secretion, rendering a more homogeneous pharmacologic response in noninbred animals, thus being an effective experimental procedure.

P223 Does the Handling Frequency during the Acclimatization Affect the Reaction of DA Rats to Routine Experimental Procedures?

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A number of studies reported that the habituation to handling is associated with a reduction in stress response and such habituation can decrease the effects of stress associated with human interactions. Thus regular handling during acclimatisation can be beneficial for the welfare of laboratory animals and reduce the influence of stress response on experimental results. Due to the limited information about the influence of handling frequency the present study focused on the effects of handling method, duration and frequency performed during acclimatisation. After weaning the DA rats were randomly allotted to 4 groups, in groups of two or three per cage. Group G5 and G1 were gently stroked for five (G5) or one minutes (G1), Group S was tail-lifted and Group K was lifted by grabbing the chest without any further contact to humans. Rats were handled either twice a week (Experiment 1, 20 males, 18 females) or five days a week (Experiment 2, 23 males, 24 females) for

three weeks (4-6 weeks of age). At seven weeks of age the behavior of each rat was observed during routine experimental procedure and recorded by experienced and inexperienced personnel (double blind test). Handling methods showed an effect on the reactions of rats to routine experimental procedure, especially to the human-contacts. Increasing the handling frequency led to a decreased anxiety in Group S and K and reduced the aggression of Group S during restrain procedure. Prolonging the handling duration from one minute to five minutes didn't have an effect on anxious reaction.

P224 The 3Rs Implementation in Practical Works: Results from a 7-Year Survey of FELASA Accredited Course at the National Veterinary School of Toulouse

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The FELASA training course 011/05, organized at ENVT was accredited on 14 February 2006 as C level. This course is entitled "Use and care of laboratory animals", ie "Utilisation et Protection des Animaux de Laboratoire" (UPAL). Based on the FELASA annual reports, the main features characterising this course will be summarized and analyzed, focusing on the implementation of the 3Rs in practical works. UPAL organises two annual sessions. From 2006 to 2012, a total of 473 students attended the courses, with 243 and 230 students in March and in September, respectively. They included doctoral or post-graduate scientists from Toulouse University, but also from other Universities. Continuing education candidates represent the major part of students, comprising researchers from public and from private institutes. The staff included 17 teachers, half coming from private or public institutes; the other half from Veterinary Schools (Toulouse and Alfort). Electronic evaluation forms, asking general questions but also rating each presentation on the pertinence of the subject and on the quality of the presentation allowed fine analysis of the feed-back of each course. For wet-lab practice, students were divided in two subgroups (composed of 15 to 20 students each). The improvements of practical works included- Reducing the number of animals per student- Replacing animals by using inert device for stitches training- Refining animals use, by switching from injectable to inhalation anesthesia and morphine analgesia- Re-using animals for other pedagogic or scientific purposes instead of using another set of animals

P225 Choosing the "Best Monkey" and Training It for a Diabetes Research Project

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According the 3Rs rules, research projects gain advantages by training of nonhuman primates (NHP) to cooperate with the research team. The training of NHP facilitates the experimental procedures and makes it cheaper. Every study done with trained primates benefits animal welfare, reduces stress, avoids the need to use chemical restrain, and helps to get scientifically better results. Our experience along the last years shows that monkeys are not all the same as to the training performance, and we have developed a list of criteria which helps to select the "best monkey" to be trained for a specific procedure. The goal of the present study was to select the "best animal" for a research project on diabetes that involves multiple interactions with the animal and a high number of daily blood testing and injections (12). We will show the different phases of training, which lasted for more than 10 weeks, and will describe step by step the training process which lead at the end to a situation where the animal will cooperate with all needed in the study. By choosing the "best monkey", the training period was shorter, easier and cheaper. Animals learned to cooperate with 8-10 glucose testing and 3 insulin injections per day for extensive periods. Same system is being implemented for choosing right animal for brain research.

P226 Reduction and Refinement in the Embryology Laboratory: Quality Control of Processes and Results

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Assisted reproductive techniques are now used at almost all steps of production and management of genetically engineered models. In particular, sperm freezing and embryo freezing are important tools to decrease the housing of live, unused, transgenic models. Knowing that the vast majority of the >6000 transgenic lines remained unused for years, this is a major point of reduction and refinement of animals used in research. This also highlights the need of efficient techniques to decrease embryo/sperm donors animals and to guarantee future revitalization of germplasms. It steers us to implement a quality control program of processes and results all over the activity. The sampling and control technique impact dramatically the representativeness of results. In house, the full program imply quality control of all media and products via mouse embryo assay (cut off >80% blastocyst production rate), regular quality control of each technician skills (>80% frozen embryo survival rate, >30% birth rate after embryo transfer, >30% IVF rate with frozen sperm) and day to day recording of parameters. This also allows us to have reference parameters to control the course of an embryology project.

P227 Long-Term Follow-Up of Adaptiveness of Laboratory Beagles Adopted as Home Pets

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Some dogs used for laboratory investigations can be adopted by a family whenever euthanasia is not required. Whether these dogs are able to adapt themselves as pets has to be investigated. Our laboratory has set up an adoption program for adult Beagle bitches, once neutered for experimental purposes. This study was designed to evaluate the behavior of the animals after their adoption. Over a 9-year period (2002 to 2010), 191 bitches have been adopted. A total of 105 families were then contacted by telephone and asked to answer a questionnaire. The questionnaire included information about the family, its environment, and questions to evaluate how the dogs adapt to their new environment. Two consistent phases were observed following adoption. First, dogs went through an initial acute phase of stress that included anorexia, resistance to urinate or defecate outside, and reluctance to walk on leash. The second phase shows dogs's adaptation. The dogs became clean, were eager to be walked and became friendly with family members and other animals. At the time of the survey, 92% of those dogs were reported as clean, 84% were cudly, 89% were demonstrative and 84% were fully adapted to their new environment, although still easily frightened (84%). Yet, most adopting families expressed satisfaction (8.8/10). Looking back to the behavior of dogs in the laboratory yielded a pretty good indication of their future adaptiveness. This adoption program can be regarded as successful and the rehabilitation of laboratory dogs turned out to be a desirable alternative to euthanasia.

P228 Influence of Housing Conditions on Behavior and Recovery of Female Mice Following Surgery

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The transportation of laboratory mice into a new clean cage as well as short-term individual housing are standard procedures after surgery but may increase vulnerability to surgical stress or interfere with postsurgical recovery. To analyse the effect of postsurgical housing, female pair- and single-housed C57BL/6J mice in their familiar home cage or single-housed mice in a new, clean cage after minor surgery ± analgesia, anesthesia only or no treatment were monitored using non-invasive methods during the immediate postsurgical period to assess pain and general impairment. Behavioral rhythmicity was disrupted, and behaviors related to wellbeing, such as nest building and burrowing decreased after experiments. Social interaction in pairs was nearly absent. Burrowing latency ranged from an intermediate level following anesthesia only and surgery with analgesia, to pronounced prolongation after surgery without analgesia in animals housed in their home cage. Significantly longer burrowing latencies after surgery without analgesia was found in individually housed animals compared to pairs. In new cages burrowing latency in general was prolonged dramatically after experiments. General activity and climbing behavior increased more strongly in new cages after treatment, leading to significant interactions between housing and treatment conditions. The behavioral differences between the postsurgical housing conditions may be interpreted as signs of reduced wellbeing, agitation and restlessness. They may indicate that mice cope better with surgical stress when housed in groups and/or in their familiar environment. The transport to a new cage and individual housing may therefore be additional stressors after an exhausting event and may affect recovery.

P229 Repeated Jugular Catheterization of Minipigs Using Ultrasound Guidance

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Serial infusion and blood sampling are often important technical aspects of an experimental design. Compared to other laboratory species like the dog or the rabbit, superficial vessels in the minipig are not readily accessible. Although minipigs have a convenient size for handling, restraint and venipuncture can be stressful and affect blood parameters. Therefore, when experiments require frequent blood sampling, catheterization is often the best option, both ethically and scientifically. Furthermore, the ear veins in minipigs are too small for placement of a catheter of adequate size for withdrawal of large sample volumes or for a dual lumen catheter allowing both infusion and sampling. This current study included several shorter periods of blood sampling/infusion. In the intermediate periods the pigs were fed a diet, and no blood samples were needed, hence the option of a totally implantable venous access system was deselected. When implanting dual-lumen catheters in the jugular veins of minipigs, we used the Seldinger Technique assisted be the use of ultrasound guidance. We found that this approach is a refinement, as it provides a relative easy and safe method to locate and puncture deep veins, avoiding a more invasive and traumatizing surgical approach. Once the catheter is withdrawn, the vessel is left patent, enabling for several catheterizations during the study period. In certain types of studies, this can eliminate the need for permanently placed catheters, allowing periods of group housing and decreasing the number of animals excluded from the study prematurely due to complications related to long term catheterization.

P230 Use of Propofol during Endotracheal Intubation in Rats

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The endotracheal intubation in rats allows the maintenance of the airways during several surgical models. Cocktails of ketamine and xylacine are used to perform the intubation and to facilitate the animal handling previously. Moreover, propofol is widely used in human clinical practice for endotracheal intubation because of its anesthetic and muscle relaxant effects. We studied the propofol effects during endotracheal intubation by comparing two groups of male wistar rats (10 per group) with a average weight of 355 g and 346 g. They were treated with a mix of ketamine (80 mg/kg) and xylacine (10 mg/kg) and the same cocktail in addition of propofol (80 mg/kg) respectively. All drugs were administered with intraperitoneal injection. In control group, one attemp was necessary to get a usefull cannulation in seven rats, two attemps for two rats and one rat was no possible to be cannulated may be due to the presence of a deglution reflex in these animals. Endotracheal intubation was usefull in 90% of rats in the second group, in which only on rtas was cannulated in a second assay due to deglution reflex. The control gruop had a lower muscle relaxation showing a higher resistance for airways cannulation when the guide was inserted. Finally, the anesthesia times were thre fold higher for propofol group than control group. There were no differences in mortality rates. Propofol in adittion of ketamine/xylacine cocktail could improve the intubation procedure and increase the anesthesia times without any vital risk for rats. However, more subjects should be studied for concluding results.

P231 Replacement of Rodents with PCR for Infectious Agent Screening

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In addition to rodents used for research, additional rodents are used to monitor biologic materials or study rodents for infectious agents. Virus PCR assay panels have replaced the majority of rodent use for antibody production tests. To accommodate large numbers of virus PCR panel submissions and assays, we integrated a high-density qPCR array and thereafter used this same platform to develop a PCR rodent infectious agent (PRIA) array to screen production barrier rooms and isolators for reportable infectious agents. Subsequently, we investigated PRIA as a quarantine alternative to sentinels by using pet shop mice to simulate a quarantine scenario. CD-1 contact and bedding sentinels were screened by traditional methods and PRIA at 4, 8, and 12 wk (n = 3 mice/time point) post exposure. Fecal pellets, fur/perianal hair swabs, and oral swabs were pooled at day 0, 1, 2, 3, 4, and 7 for each of four pet shop mouse cages and analyzed by PRIA. Of the agents present in the pet shop mice, *M. pulmonis, P. pneumotropica,* and *Giardia* were not detected in contact sentinels. Additionally in bedding sentinels, *Spironucleus*, *Cryptosporidium*, and adenovirus were not detected and fur mites and *Helicobacter* were poorly detected. PRIA detected all agents at early time points. These results demonstrate that the direct testing of quarantined rodents by PCR can replace sentinel rodents, improve detection, and reduce the quarantine time. Preliminary data also demonstrated that infectious agents can be detected in exhaust air samples from ventilated racks suggesting that PCR could potentially be used to reduce bedding sentinel use for routine health monitoring.

P232 Antibody-Based Multiplex Assay for the Comparison of Cytokine Levels in Serum, EDTA Plasma and Heparinized Plasma Quantified from Male NMRI Mice

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Antibody based multiplex assays enable researchers to quantify multiple analytes from small sample volumes. This method is suitable for gaining the most information of small blood samples, obtained from for example, small laboratory rodents. The Bio-Plex Pro Assay has been validated for mouse serum and EDTA plasma. However, it remains to be

investigated whether heparinized plasma is suitable for cytokine quantification using this method. As heparin is a widely used anticoagulant in animal experiments, this study aimed at validating the multiplex assay for heparin-plasma. A panel of 23 acute phase and pro-inflammatory cytokines was quantified from serum, EDTA plasma and heparinized plasma from male NMRI mice. The objective was to identify specific cytokines, whose concentration in heparin-plasma were significantly different from serum and EDTA plasma. This was investigated firstly on physiologic values of unstressed and stressed mice and secondly on samples spiked with cytokine standards and with three concentrations of heparin in order to investigate whether heparin in increasing concentration had an effect on the recovery of cytokines. In general, there was no difference between anticoagulants. On physiologic values, five out of twenty-three cytokines were significantly different between groups. In cytokine-spiked samples, there was a significant difference between anticoagulants concerning eight cytokines. The cytokine recovery could be correlated either linearly or exponentially to the amount of heparin. Based on these results, we conclude that heparin is an equally suitable anticoagulant for obtaining plasma for antibody based multiplex assays.

P233 Development of a Device to Standardise and Facilitate Intravitreal Injections in Rabbits

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Treatment of degenerative diseases of the eye requires the medicine to be injected directly into the eye (intravitreal injection). Rabbits are often the species of choice in pre-clinical ocular research because of their widespread use in toxicology. The large lens and the small pars plana are anatomic features of the rabbit eye which make the injection challenging. Damage to the lens and the retina is a commonly observed side effect. Photographs, MRI-, Histology- and Laser Scanning Ophthalmoscope images, which illustrate these side effects, will be presented. To refine the intravitreal injection technique in rabbits we are currently designing a device which fixes the angle and depth of the injection. Our hypothesis is that the device will allow safer and more accurate injection into the eye than the traditional technique. In a comparative study anaesthetized New Zealand White rabbits will be injected into the eye with a test compound either using the new device or injecting in the traditional way. To establish the safety of the device ophthalmic examinations are going to be performed on day 3, 5, 7 and 14 using a slit lamp, a direct ophthalmoscope and laser scanning ophthalmoscope. At the end of the study eyes are going to be removed and analyzed for drug levels in the vitreus, which will inform us about the accuracy of drug delivery. This project is funded by the NC3Rs.

P234 A Global Pharmaceutical Company Initiative: An Evidence-Based Approach to Define the Upper Limit of Body Weight Loss in Short-Term Toxicity Studies

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Short term toxicity studies are conducted in animals to provide information on major adverse effects typically at the maximum tolerated dose (MTD). Such studies are important from a scientific and ethical perspective as they are used to make decisions on progression of potential candidate drugs, and to set dose levels for subsequent studies. The MTD is usually determined by parameters such as clinical signs and reductions in body weight and food consumption. However, these assessments are often subjective and there are no published criteria to guide the selection of an appropriate MTD. Even where an objective measurement exists, such as body weight loss (BWL), there is no agreement on what level constitutes an MTD. A global initiative including 15 companies, led by the National Center for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) has shared data on BWL in toxicity studies to assess the impact on the animal and the study outcome. Information on 151 studies has been used to develop an alert/warning system for BWL in short term toxicity studies. The data analysis supports BWL limits for short term dosing (up to 7 days) of 10% for rat and dog and 6% for nonhuman primates (NHPs).

P235 Intraperitoneal Anesthesia with Alphaxalone in Combination with Dexmedetomidine and Fentanyl in Rat

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In a previous pilot study we have determined a lack of analgesia following the intraperitoneal administration of the anaesthetic alphaxalone at various doses. Then we hypothesized that the co-administration of dexmedetomidine and fentanyl would increase analgesia reducing alphaxalone doses. In a prospective, randomized, crossover, experimental study, 32 Sprague–Dawley adult rats were administered alphaxalone (males: 75mg/kg, $n = 8$; females: 25mg/kg, $n = 8$) with dexmedetomidine 50µg/kg or alphaxalone (males: 60mg/kg, $n = 8$; females: 20mg/kg, $n = 8$) with dexmedetomidine 50µg/kg and fentanyl 0.1mg/kg. Times to loss of the righting reflex, loss of pedal withdrawal reflex, and duration of anesthesia (loss of righting reflex), and basic cardiorespiratory data (heart and respiratory rates) were recorded. The combination of alphaxalone-dexmedetomidine produced the loss of pedal withdrawal reflex and lasted longer in females (males: 25 ± 5 minutes, females: 37 ± 5 minutes) although no differences were observed between sexes when fentanyl was co-administered (51 ± 14 and 50 ± 16 minutes, males and females, respectively). Both alphaxalone-dexmedetomidine (males: 133 ± 6 minutes, females: 119 ± 13 minutes, $P < 0.05$) and alphaxalone-dexmedetomidine-fentanyl produced longer duration of anesthesia in males (males: 140 ± 15 minutes, females: 116 ± 8 minutes, $P < 0.05$). These differences have been attributed to different concentrations of estrogens between sexes. Duration of anesthesia was variable among individuals and the use of an α-2 antagonist may be considered. We concluded that the combination of alphaxalone and dexmedetomidine, with or without fentanyl, provided anesthesia characterized by loss of pedal reflex potentially suitable for surgical procedures in rat. However, differences in the anaesthetic effect observed between males and females may limit the use of alphaxalone.

P236 An Assessment of the Efficacy of Anaesthetic and Analgesic Regimens involving Rodents in Germany

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According to Directive 2010/63/EU all Member States must *"ensure refinement [...] of methodsused in procedures, eliminating or reducing to the minimum any possible pain, suffering, distress or lasting harm to the animals."* (Art.4). This project was initiated to appraise the current situation of Refinement in Germany in order to identify where improvements should be made. Animal research applications from all over Germany of 2010 were examined to assess the refinement methods used in laboratories throughout the country. The study focussed on applications involving rats and mice as they are the most commonly used species. The project aim is to assess the efficacy of proposed anaesthetic and analgesic regimens by examining applications in which rodents undergo surgical procedures. The study was carried out anonymously. Results of project applications reviewed to date show that Isoflurane was the most widely

used inhalant anaesthetic in both rats (34%) and mice (32%). Isoflurane was either combined with an analgesic or used as the sole agent (rat: 30%; mouse: 24%). Ketamine/xylazine was the most frequently used injectable anaesthetic combination (rat: 28%; mouse: 31%). Multimodal analgesic regimens were rare (9%) and were considered by experimenters only in exceptional cases following severe surgeries. In 46% of the applications the researcher's severity classification differed from the classification according to Annex VIII of Directive 2010/63/EU and other pain catalogues. Thus, the postoperative administration of analgesics did oftentimes not correlate with the severity of the experimental procedure, and the perioperative preventative use of analgesics was not consistently used.

P237 Authentication and Quality Control Guidelines for Human Transplantable Tumors and Cell Lines

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Human transplantable tumors are used to model human cancer in laboratory animals. Human cell lines are used in research studies as both a replacement to the use of laboratory animals as well as reduction of laboratory animal use by prescreening in cell culture prior to initiating laboratory animal studies. However, it is increasingly recognized that cell culture and transplantable tumor misidentification or contamination occurs frequently. Erroneous data using incorrect or contaminated materials may actually lead to increased use of animals due to the need to repeat studies and determine the cause of confounding research results. We performed a retrospective study examining the number of human cell lines and transplantable tumors submitted to our laboratory that had erroneous genetic profiles indicative of genetic contamination. We found that 3.4% of the human samples examined contained cell lines of another species. Mouse cell lines were the most common contaminant, followed by rat and Chinese hamster origin cells. In addition, we found that 6.9% of the human samples were contaminated with another human cell line. When we excluded samples submitted by companies with stringent monitoring and quality control programs, we found that 23% of the samples submitted were contaminated. These findings illustrate the critical need for quality control programs when using cell lines and transplantable tumors. This presentation will focus on the components of a quality control program for cell lines and transplantable tumors. Testing guidelines and best practices to prevent contamination will also be covered.

P238 Implementation of the 3Rs: Optimization of a Breeding Protocol in Mice

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Nowadays, the breeding design optimization is a priority for reducing the number of animals used in the maintenance of different strains. Thus, the optimization of mice breeding programs is really needed to obtain a high crossover success. We designed and tested a series of breeding protocols in which we evaluated different parameters that are directly related to the predisposition of mice for mating. In the present work, we used C57BL/6 mice. We tried out different crossing models (trio, group, harem), number of days for acclimation period, days of cycle synchronization, different methods of male housed and methods of induction of estrus (whitten effect). The success of mounts was proven by the detection of vaginal plugs, certified lately with the development of late gestation. Our data demonstrated that the optimal crossover model was one male with two females. In the best breeding protocol: a males was housed individually for 7 days, period in which no changes were made in the bed of the cage; two females stayed a minimum of 15 days together for the synchronization cycle; the bed and hair coming from the males was mixed with the bed of the cages of females during 72 h. In this crossover units, mice were kept together up to 96 h maximum; during this period, we checked the presence of vaginal plugs twice a day (early in morning and late at the afternoon) and when a vaginal plug was found, we individualized the female. These parameters provide an effectiveness rate of 60% for mating.

P239 SAMSEI Project – Implementing the 3Rs on the Human Patient: Surgical Skills Training

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The poster will present the SAMSEI project (Stratégie d'Apprentissage des Métiers de la Santé en Environnement Immersif) developped by the Claude Bernard Lyon 1 University (UCBL). It's one of the three projects in Lyon selected by the IDEFI framework, organized by the Ministry of Higher education and Research. The goal is to set up teaching programs based on simulation; the project will offer a realistic and interactive training to the students of all health sectors of the UCBL and its partners. It's actually carried out by Pr. MARTIN Xavier (School of Surgery Director - UCBL) in collaboration with the UNF3S (Université Numérique Francophone en Science de la Santé et du Sport) of Lille et the University Val de Grâce of Paris. During their training, the students learn surgery step by step. They start in a 3D animation simulator (supported by ICAP), then they move on low and high fidelity mannequins (support by Médiathèque) and finally, they complete their training on pigs (School of Surgery). From an anatomic point of view, the pig is the closest animal to man. At the School of Surgery, we have developped celioscopy educational courses on pigs. Moreover, we have a training course in microsurgery in wich we use both substitutes (rat artery, double-clock) and animals. At the end of the training, the animals' body parts are harvested and uses in the Basic Surgical Skills training, wich is done in collaboration with the Royal College of Edinburg.

P240 The Effect of Tramadol Induced Analgesia on Mice Undergoing Embryo Transfer

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Mouse embryo transfer is broadly used in transgenic mouse production or for rederivation purposes. Although embryo transfer is considered as an invasive procedure, the administration of a pain relief is not considered necessary -along with the extra expense- or because it is believed that the drug will produce adverse effects. The aim of the study was to evaluate the effects of tramadol, a synthetic opioid, on the recipient dam and on the transferred embryos. Twenty-six Crl:CD1 female mice, body weighted between 25 to 35 g were divided in two groups. All animals were anesthetized using (ketamine/xylazine 0.07 mL/g). A single subcutaneous dose of tramadol (20 mg/kg) was administered perioperatively to mice of group A (n = 13) while mice of group B (n = 13) didn't received any analgesic treatment. Body weights, birth rate, number of offspring weaned per dam and weight of pups were evaluated. All mice of group A carried embryos to term, whereas 10 out of the 13 dams of the control group carried embryos to term ($P > 0.05$). No significant difference was seen in the surrogates' body weight or the birth rate ($P > 0.05$). The yield and bodyweight of pups showed statistical significance with increased rates for the tramadol group ($P < 0.05$). In conclusion, tramadol can be used for pain management of mice underwent embryo transfer without any side effect while there is a positive influence on the yield and body weight of pups.

P241 Capillary Microsampling of Plasma in Rodent Safety Studies

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A novel blood sampling method (capillary micro-sampling, CMS), has been developed by AstraZeneca for the determination of pharmaceutical drugs or metabolites in plasma using just 32 µL of blood per sample. The fast and easy collection of an accurate sample volume, together with the liquid matrix makes the technique applicable to safety studies conducted in rodents where analysis of pharmaceutical drug exposure is a regulatory requirement. The small sample volumes allow toxicology endpoints and pharmaceutical drug exposure to be evaluated in the same individual animals as it is currently standard practice in larger animals. The use of additional animals (often referred to as satellite animals) solely for toxicokinetic analysis is routine practice in rodent safety studies and therefore the introduction of CMS reduces the use of animals in these studies. The small sample volume means less invasive sampling procedures in adult animals and also makes it possible to collect samples from juvenile animals. Safety studies are laborious and resource demanding including daily dosing, observation, husbandry and care of individual animals. Thus, in addition to animal number reduction, the refined sampling methods and the increased scientific value from studies, the method leads to significant cost reductions due to reduced animal work and amount of compound needed.

P242 Automatic Cognitive Testing in Social Groups in Macaques

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Primate cognitive behavior in the laboratory has often been evaluated by housing subjects individually or isolating them, and by imposing fluid or dietary restrictions to increase the subject's motivation to work. Advances in animal welfare have significantly changed the way in which research institutions house primates in terms of space and numbers, accompanied by enrichment programs with novel objects and food that break with traditional feeding habits. Although some could potentially see these changes as a bias to previously published data, others have already proved that it is possible to obtain remarkable scientific results while offering primates a highly enriched environment. Inspired by recent publications on automated cognitive testing in social groups, our laboratory developed a special application on tactile screens, AUTOBUNTO, by which each primate learnt its own pin code to launch a single trial of its own behavioral test. This system allows testing animals on different cognitive tests while preserving social groups in their home cages. Two tactile screens can be installed at two ends of the gang cage to avoid dominance issues over screen availability. Results suggest that gang-training to touch tactile screens is quick and that completion of different cognitive tests, including visual discrimination tasks and working memory, can be acquired in a few weeks. More importantly, primates are free to work whenever they desire it instead of being imposed with a rigid testing schedule. Finally, isolation or dietary restrictions seem unnecessary for healthy primates to perform cognitive tests on tactile screens.

P243 Directive 2010/63/EU: A Chance for More Humane Education?

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Recital 10 of Directive 2010/63/EU states that "this Directive represents an important step towards achieving the final goal of full replacement of procedures on live animals [...]. To that end, it seeks to facilitate and promote the advancement of alternative approaches.". Recital 12 directs that "The use of animals for [...] educational purposes should [...] only be considered where a nonanimal alternative is unavailable". Thus, the potential benefits for animals are huge. However, the degree to which contemporary educational practices involving animal use will be altered remains to be seen. Since more than 10% of the animals used annually for teaching purposes within Germany are used in Berlin, 30 Berlin-based educational programs from 2012 were reviewed. In 42% of the programs alternative teaching methods were used to complement and reduce the use of live animals - primarily by using multimedia software prior to teaching with live animals. However, in more than 90% of the programs further implementation of the 3Rs seemed feasible. Some teachers mentioned that they could not teach with alternative methods first, because all the available time was needed for the animal-based exercises. A solution to this problem could be the mandatory establishment and use of so called AltLabs – laboratories containing various alternative teaching materials – in every research center. These AltLabs must be equipped with competent instructors and have adequate opening hours so that students can receive sufficient training prior to their work with live animals.

P244 Maternal Weight as an Alternative Determinant of the Gestational Day of Wistar Rats Housed in IVC Cages

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The laboratory rat is an animal model widely used in fertility, developmental and toxicity studies. In many of these the early recognition of pregnancy as well the confirmation of the exact embryonic day are indispensable. The purpose of this study was to look into the possible correlation of maternal weight at the time of conception and its increment throughout the gestation days, in order to have a good baseline to determine and monitor pregnancy from the onset.132 female HsdOla:WI (Wistar) rats were chosen with only selection criterion their body weight (nulliparity and age were not examined) and taken into the male's cages for 72 hours. As D0 we were considering the day in which copulatory plug or sperm was found in the vaginal smear examination. Then the animals were transferred back to their cages and weighting started 14 days after D0 until the time of delivery. From the statistical analysis appears that there is no correlation between the litter size and the weight of the pregnant animals from day 14 to day 19 of gestation. In contrast, there is a correlation between the initial maternal body weight and the weight of the pregnant animals on the same days. Finally, it was observed that the average weight gain from day 14 to day 19 is positively correlated with the initial body weight of the animals. In conclusion, body weight measurement can be used for the determination of the gestation day in rats.

P245 Minimally Invasive Central Venous Catheters in Obese Minipigs Allows Marked Refinement of the Model and Reduction in the Number of Pigs Used

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Obese Göttingen minipigs are used as a model of obesity due to the similarities to obese humans. Typically the same animals are used for several years and during the studies there is a need for taking regular blood samples. The gross obesity and the lack of easily accessible veins prevent easy and stress-free blood sampling, and therefore the method of choice has been an invasive placement of central venous catheters. These tend to have a limited period of use due to catheter-related infection/thrombosis. Therefore a new minimally invasive method has been evaluated. In lean, male Göttingen minipigs catheters were placed either in v. jugularis through the ear vein using a minimally invasive technique or in v. cava caudalis through v. cava cranialis using a more invasive technique (n = 12). The catheters were evaluated over 8 weeks

with respect to functionality and pathologic changes. In addition, the ear vein catheters were tested in grossly obese minipigs and their functionality was compared to historical data from the invasive method ($n = 18$). Minimally invasive catheters placed through the ear vein in Göttingen minipigs lead to only minor catheter-related pathologic changes ($P < 0.001$ compared with the more invasive method), the catheters could in most cases be removed and replaced again and their functionality in both lean and obese minipigs was similar to that of the invasive method. In conclusion, the use of these catheters refines the obese minipig model and potentially reduces the number of obese animals that have to be euthanized prematurely due to catheter related problems.

P246 A New Method to Analyze the Corticosterone Concentrations in the Hair of Rats and Its Application to the Study of Chronic Stress

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The exposure to chronic stressors could have deleterious effects on an animal's health and wellbeing and could negatively influence the result of an experimental design. The stress situation involves an activation of the hypothalamic–pituitary–adrenocortical (HPA) axis changes in the adrenocortical secretion of glucocorticoids (GC). Short-term and long-term changes are nowadays assessed by measuring GC concentrations in plasma, feces, urine and saliva but there are clear limitations to each of these approaches. Therefore there is no current noninvasive validated procedure for determining long-term activity of this system in laboratory animals rodents. The aim of the present study was, for the first time, to develop and validate a reliable method for measuring hair corticosterone concentrations in rats. Sprague–Dawley rats of 10 weeks were shaved in the morning on their back and collected 200 mg of hair samples. The procedure involved: two isopropanol washes of the samples to remove contaminants, powdering of the washed and dried hair, a 21-h methanol extraction followed by evaporation of the solvent, reconstitution of the extract in phosphate buffer and analysis of the extracted corticosterone by radioimmuneassay (RIA). The sensitivity of the assay was 0.6 pg/mg. The measured recovery was: 97.5% ± 6.1 and the coefficient of variation of the whole process (extraction + analysis): 7.8%. Therefore this new procedure could be useful in many experimental contexts involving the study of stress, endocrinology and neuroscience fields but also for monitoring chronic stress that might be associated with experimental manipulations.

P247 Assessing Cryopreserved Samples of Mutant Mice

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Genetically modified animals are unique mutants with an enormous scientific potential. Cryopreservation of pre-implantation embryos or of spermatozoa is a common approach to save those lines. The breeding of a line can be discontinued if a sufficient number of assessed samples is cryopreserved. Several assessments are available, possibly with a loss of material. Regenotyping of the donors is helpful. Embryos: At least one specimen must be revitalized and transferred into foster-mothers. If the genotypes of the litter are not as expected, the reason has to be identified; additional embryos must be cryopreserved. In vitro-cultures demonstrate the developmental capacity. Spermatozoa: The in vitro-fertilization (IVF) capacity of each line must be demonstrated. Each donor should be assessed. To circumvent the animal consuming IVF we developed a fluorescence microscopy based, animal-free technique analyzing the membrane integrity. Physical and hygienic stability: Samples of the same line should be protected against accidental loss by storage in different permanent freezers at several locations. To ensure long time storage sample should be stored in the liquid phase of LN_2, otherwise the temperature stability must be monitored. A powerful data management is important. We never found microbiological contaminations of a sample within a cryo-tube. However, environmental organisms were found frequently in the freezers. Since contaminations cannot be finally excluded and an embryo-transfer might not lead in all cases to a secure rederivation, foster-mothers and revitalized pups should be housed in an intermediate facility and health assessed before introducing into the target facility.

P248 You Do Not Keep Anything Clean without Getting Something Else Dirty! Care of the Nude Mouse as a Sentinel Strain in a Nonsterile IVC Environment

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IVC cage systems are widely used in many rodent research facilities. Traditionally these units are monitored using immunocompetent sentinels exposed to dirty bedding and tested via methods including serology, bacteriology and parasitology. This screening method can be costly and requires the use of many animals. Also, because various factors may influence detection, it is not always effective. Our poster demonstrates how the use of immunocompetent sentinels is partially replaced by introducing immunodeficient sentinels where samples are tested via PCR. Whilst there may not be any advantage with regards to the transmission to the sentinel cage, there is an increased susceptibility of the sentinel to pathogens. We focus on the use of the nude mouse as a sentinel housed in nonsterile conditions over a 6 month period in a large academic establishment, and the husbandry and care provided by animal technicians. The nude mouse is vulnerable to a variety of opportunistic infections; however there are few reports of maintaining this strain over an extended time and none which demonstrate deliberate exposure to pathogens via dirty bedding in a nonsterile environment. Data presented will summarise the strict housing and checking regimes, health recording, weight records and incidence of spontaneous disease. These protocols have been reviewed and approved by our establishment's ethics committee. It supports the theme of FELASA's Congress "Better Science from Fewer Animals" and the 3Rs, as there is an overall reduction in animals used for routine health screening and avoidance of stress associated with live animal transport.

P249 Landmark-Guided Intra-Articular Dosing and Sampling in the Cynomolgus Macaque

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Cynomolgus macaques are commonly used in research; however few reports describe the femoropatellar and femorotibial joint structures and techniques for intra-articular (IA) injection and synovial fluid sampling. Human literature reports variable injection accuracy, and proposes ultrasound guidance for injections. This investigation determined the articular and synovial structure in the Cynomolgus macaque and developed an approach to reliable IA injection and sampling using landmark guidance. Anatomic structure and IA injection accuracy were evaluated with contrast radiographs and latex injection of cadaver joints. These revealed a noncomplex synovial structure without separate joint pouches as described in other species. Anatomic landmarks were determined for injection and sampling. Cadaver knees ($n = 30$) were injected with radiologic contrast and/or latex. Using the anatomic landmarks, IA injection accuracy was 100% (30/30 joints). Minimal leakage from the needle track was noted in 2 joints. Following technique development, nine macaques (5 male, 4 female; 3.3-8kg; anesthetized) underwent repeated IA injection and aspiration of lactated Ringer's solution (LRS) of one joint weekly for three weeks. Animals were closely observed for 7 days after injection. No swelling, lameness, or other adverse effects were noted. Aspirates collected were consistent with normal joint fluid

and LRS, with recoveries of 30-60% of initial injection volume in 26 of 27 joints. The IA injection and sampling technique developed is accurate and does not require specialized equipment, and fewer animals are needed with proper technique and training.

P250 Suitability of Submandibular Venipuncture, over Retroorbital Sinus Sampling, for Vaccine Research Studies in Mice

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In vaccine research protocols using mice, blood is frequently collected to assess immune responses, most commonly through retroorbital sinus bleeding. However, because retroorbital blood collection can result in severe orbital tissue damage or possible blindness, the use of this method is controversial. In order to promote a more humane procedure, we assessed the reliability of blood collection from the submandibular vein in comparison to retroorbital sinus sampling in mice. To do so, we compared the time required for blood collection, the quality of collected blood samples, the results obtained from different immunologic assays, and the users' safety. CD1 females between the ages of 3 to 4 months were all bled by the same experienced technician. Blood was collected 2 weeks apart in the same animal using either method. An average of 30 seconds was required for the blood collection through both methods, but the need of anesthesia added 90 seconds to the retroorbital sinus sampling procedure. Bacteriology testing of serum did not reveal any signs of contamination of samples obtained through either technique. Ongoing experiments will determine fungal contamination. Preliminary results indicate no significant difference between samples when used for virus neutralization assay and binding antibody assay by ELISA. Finally, recommendations will be made to increase investigators' safety in the use of submandibular venipuncture when used in animals infected with virus. In conclusion, we consider the submandibular venipuncture as an adequate and more humane alternative to retroorbital sinus bleeding for mice used in vaccine research studies.

P251 The Zebrafish Embryos as an Alternative Approach to Anaesthetic Animal Research

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In research, rodent species are the predominant animal models used. Although, the new directive 2010/63/EU establishes the implementation of ethical principles that value the animal welfare as well as the number of animals used. In this context, the use of zebrafish embryos is receiving increasing attention as they are not covered until the point they start feeding independently (5 days post-fertilization-hpf). The purpose of this work is to take advantage of the benefits of zebrafish embryo assay and to predict the teratogenic potential of ketamine, a commonly used anaesthetic in rodents. Between 2-3 hpf zebrafish embryos were exposed during 20 minutes to ketamine (0.0, 0.2, 0.4 and 0.8 mg/mL). Ethanol 2% was used as a positive control. Morphologic parameters and overall pattern of cell death were analyzed to assess lethality and/or developmental anomalies based on specific time endpoints until 144 hpf. Results showed a concentration-dependent increase in anomalies and mortality. Cephalic disorders, enlarged organs and tail/spine anomalies were the most prominent deformities observed at 144 hpf. In conclusion, 20 minutes of ketamine anesthesia with sub to over anaesthetic doses during early developmental phases of zebrafish interferes with the normal developmental pathways of zebrafish embryos causing several anomalies that may result in long-term disabilities at later developmental stages of zebrafish. These effects were already observed when young higher vertebrate animals were exposed to anaesthetic agents causing widespread apoptosis and abnormal behaviors and thus validates the use of zebrafish embryos as an alternative model for anaesthesiology research, according to the 3Rs principle.

P252 A Novel Method of Rabbit Endotracheal Intubation Using Endoscope: Comparative Study of Endoscope and Laryngoscope for Training

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Endotracheal intubation in rabbits is technically difficult due to their oropharyngeal anatomic features and a numerous techniques and devices have been described. No visualization of the epiglottis and vocal cords results in repeated intubation attempts which can cause damage to the epithelia in the oral cavity or around the epiglottis, and death of the animal due to respiratory failure. We have published a newly developed method as ESE unit using endoscope, to overcome the obstacle in the Proceeding of the Eleventh FELASA and the 40th Scand-LAS Symposium, p185-188, 2010. In this presentation, we describe that a comparison study consists of an alternative method with a laryngoscope along with our method was conducted to reveal the usefulness and learning speed for the beginner of endotracheal intubation in the rabbit. Four male New Zealand White rabbits weighing 2.7 ± 0.19 kg were randomly allocated into two study groups (n = 2 animals each) to perform either a newly developed endoscope or laryngoscope. Twelve trainees were assigned randomly to one of two study groups which were 'laryngoscope' group (n = 6) or 'endoscope' group (n = 6). Each trainee was allowed to perform the intubation within two minutes in one attempt and to repeat three attempts. The results of comparative study revealed that ten trainees out of twelve achieved the endotracheal intubation using ESE unit within two minutes, as two trainees out of twelve using laryngoscope. The findings suggest that endoscope method can be used as an easy, less traumatic method of rabbit intubation when compared with laryngoscope.

P253 Extraction of DNA from Mice Samples: A Comparison of Quantity and Quality Performance from Different Tissues and Cells

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Genotyping of mice is a common procedure in animal facilities. The aim of this study was to compare the quantity and quality performance of DNA extraction from different samples obtained at two different ages: young mice (YM; 10 days old), and adult mice (AM; 12 weeks old). In addition, we valued some subjective parameters related to the welfare of animals and the easiness of the procedure. Both, in YM and AM, samples were collected from tail, ear and phalange. In AM, samples were also taken from hair, fecal pellets, buccal swab (BS), and blood. DNA was isolated by commercial kits and concentration and quality were measured by spectrophotometric absorbance. The DNA integrity was valued by electrophoresis on agarose gel and by PCR. DNA was amplified in all samples, although the intensity of the band was heterogeneous. Amplicons from hair and BS, gave weak bands, probably due to DNA low quality and fecal samples, gave weak bands probably due to any interference with DNA from stool microorganisms. In general, tissues from YM yielded higher DNA quantities than those from adult. For example, DNA extraction from ear samples yielded 37.77 ± 2,03 ng/µL (mean ± SD) in YM, and 7,34 ± 2,98 ng/µL in AM. In YM, the

best results were obtained from ear and tail samples, with statistically significant differences regarding phalange samples ($P = 0.035$ and $P = 0.007$, respectively). In AM, the best results were obtained from tail and blood samples, however, the tail biopsy was less painful and stressful than blood extraction.

P254 Validity of Chemical and In Vitro Analysis in Studies on the Nutritional Value of Differently Processed Rat Diets

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The objective of the study was to find out whether chemical and in vitro analysis developed for other animal species can be helpful in the evaluation of protein and energy value of autoclaved diets for laboratory rodents and if they can be recommended as alternatives to the animal tests. Two natural-ingredient diets containing either soybean or casein as main protein sources, and commercial diet, were sterilized by autoclaving during 20 minutes at 121°C or during 10 minutes at 134°C. Control diets were not sterilized. The effects of autoclaving on protein digestibility and biologic value were determined in rats and compared with amount of protein bound to fiber, protein solubility in KOH and sodium borate, and ileal protein digestibility assayed in vitro. Metabolizable energy concentration of the diets was measured in rats, determined in vitro, and calculated according to Atwater's equation or formula developed for pig diets. Except ileal protein digestibility assayed in vitro, all other chemical and in vitro analysis correlated with indicators of protein value assessed on rats. Among them, solubility in KOH and sodium borate had the highest accuracy to protein biologic value. Close agreement was found between results of rat tests and in vitro assessment of metabolizable energy content as well as validity of the pig formula based on extended chemical analysis. Obtained results indicate that there is a scope for the replacement of animal tests and improvement of the precision of predicting protein and energy value of diets for laboratory rodents. Financial support project no. NR12003506.

P255 One Team Strategy for Rodent Stereotaxic Surgery Refinement

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Stereotaxic surgery is required for the preparation of several models of neurologic disease. For example, the implantation of cannulae into specific brain regions allows investigation of the effects of test compounds directly injected into brain structures or locally manipulating neurotransmitters. The injection of specific neurotoxins aids the induction of lesions mimicking neurologic disease lesions, such as Alzheimer or Parkinson's. Electrophysiological stimulations and recording studies are facilitated by the stereotaxic implantation of electrodes. This poster presents the strategy implemented by our institution to ensure the production of high-quality and homogeneous surgically prepared rodents. The implemented strategy required technical refinements to the following: animal model selection, taking into account strain, sex and age specifications; equipment selection dependent on the animal model and surgery category; management of aseptic conditions, including environment, patient, equipment and implantable consumables; pre- and postoperative care, such as quarantine time and pain management programs; validation of procedure success to ensure high effectivity; and model development and training programs for surgery staff. This continuous improvement strategy allows our intuition to significantly increase success rates, as for example an increase from success rates of less than 40% to more than 70% for the intracisternal cannulation in rats andan increase from success rates of less than 50% to more than 80% in the Parkinson model in rats.

P256 Stress and Welfare of Diet Board Fed Sprague–Dawley Rats in a Two-Year Experiment

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In diet board (DB), food pellets are firmly embedded in grooves cut into aspen board, and rats have to gnaw wood in order to detach the food. DB makes eating more laborious, while letting the animals live in groups and be in control of when and how much they eat. A two-year experiment with Hsd:Sprague–Dawley rats, comparing DB fed males and females with corresponding ad libitum fed groups, showed that DB reduced food consumption and body weight mildly to moderately, and increased longevity significantly. In order to assess welfare, faecal corticosterone metabolites (Fcort) and IgA (FIgA) were assayed at seven time points, elevated plus-maze tests were performed at six months, and home cage behavior was analyzed at three time points. Cage behavior categories included eating, drinking, rest, agonistic behavior, escape-related behavior, grooming, and allogrooming. DB fed females had statistically significantly higher Fcort levels than other groups. Feeding groups did not differ in the anxiety-related open arm parameters of the elevated plus-maze. In the home cage analysis, DB did not affect circadian rhythms of activity and feeding, and no signs of impaired welfare were observed. During dark periods, DB fed rats spent more time eating and had more eating bouts than the controls. In conclusion, DB seems to circumvent two major problems associated with other food restriction methods, that is, incompatibility with group housing, and changed circadian rhythms; while the increase in Fcort in females can be regarded as a sign of mild to moderate stress, perhaps caused by hunger.

P257 Target Controlled Infusion in the Refinement of Rabbit Anesthesia

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Sedation and/or anesthesia of laboratory rabbits are currently required for both minor and major clinical or experimental procedures in order to reduce individual distress and suffering. The present work aims to review rabbit anesthesia with propofol and refine it by developing a pharmacokinetic/ pharmacodynamic (pk/pd) model for target controlled infusion (TCI). The kinetic variability of real propofol plasma concentrations was compared with the variability of bolus and perfusion doses which were calculated based on each animal weight. Seven New Zealand White rabbits, weighting 2.52 ± 0.11kg, were anesthetized using a propofol bolus of 20 mg/kg IV. Following blind orotracheal intubation animals were mechanically ventilated with 100% oxygen. An infusion rate of 50 mg. kg^{-1} . h^{-1} was used to maintain anesthesia during thirty minutes. Clinical data and arterial blood samples were collected at specific time points. Propofol plasma concentrations were quantified by gas-chromatography assay. For statistical analysis, means and standard deviations were used to calculate the variation coefficients (VC) for each concentration, dose and weight. As result, propofol doses calculated by weight showed low kinetic variability (VC = 4% for bolus and perfusion dose) whereas the variability among the measured propofol plasma concentrations was higher than the expected (VC from 20% to 120%). This large pharmacokinetic variability in the collected samples indicates that factors like age, sex, strain need be taken in account when performing propofol anesthesia. The development of specific pk/pd models is important to understand the source of this variability and to optimize dose calculation, allowing the implementation of precise propofol infusion with TCI.

P258 The Use of Bronchial Blockers for Selective Lung Ventilation in

the Ovine Experimental Model for Thoracic Surgery

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The ovine model for thoracic surgery is becoming widely used. The adult animal lung size and the parenchyma characteristics are similar to humans, and therefore quite appropriate to test new products and surgical techniques. One-lung ventilation is used to facilitate the surgical procedure during thoracic surgery. Selective intubation is used to maintain an adequate surgical area, keeping the ventilation of the nonoperated lung, being double-lumen tube the most usual method to isolate the lung. The ovine model for thoracic surgery has some aspects related to specie-anatomy that should be taken into account for selective intubation: the trachea length is larger than in humans, therefore disabling the use of double-lumen tubes that can not reach the bronchia by oral approach. Tracheotomy approach has been reported, but in survival models a minimal invasive approach should be preferred. Herein we describe step-by step a minimally-invasive technique that allows performing one-lung ventilation in the sheep, using an endobronchial blocker set. Under general anesthesia, oral intubation was performed in 2 sheep. The three-lumen adaptation piece was connected to tracheal tube and ventilator. Fibro-bronchoscope was introduced simultaneously with bronchial blocker, being the blocker tip directed and introduced in the left bronchus. The cuff was then inflated 5ml of air. The lung was deflated with mild aspiration, until collapse. Good animal ventilation and no adverse reactions were observed. Bronchial blocker can be used as an effective method to provide one-lung ventilation in the ovine model, adding an important improvement on animal welfare in postsurgical period.

P259 Refining Fasting Protocols in Mice

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Using telemetry, we studied the influence of fasting duration and pattern, sex, bedding material and housing in fasting mice. Hsd: ICR (CD-1) mice, weighing between 20 and 25 grams, were used (40 males and 36 females). Thirty one animals, 21 individually housed (10 males and 11 females) and 10 group-housed (females) were implanted with telemetry transmitters (TA-T20, DSI). Over a period of four weeks, each mouse was then subjected to a consecutive procedure of four fasting conditions: 6h and 12h, light and dark phase fasting (L6h, L12h D6h, D12h). Two weeks after completion of the telemetry data acquisition, we then performed gastric and cecal emptying studies and included an additional 45 CD-1 mice, subjected to the same fasting conditions. A large amount of body temperature and animal activity data were collected that deserves a thorough analysis. We found that fasting animals are more active, although a gradual descent of body temperature was observed. Different changes in body weight were found at the end of fasting (L6h= -7.06%, L12h= -9.66%, D6h= -11.29%, D12h= -16.13%). Twenty four hours after any of the four fasting conditions all animals recovered the lost weight. We always found some gastric content when wood chips bedding cages were used. None of the raised fasting patterns achieved a complete cecal emptying. Data collected in this study are of great value to design and refine new mice fasting protocols, helping to minimize the stress of the animals, ensuring gastric emptying and adapting this procedure to the workday of the husbandry staff.

P260 A Reporter Mouse to Measure Spatio-Temporally Drug Myelotoxicity and Carcinogenicity in Vivo

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Myelotoxicity and carcinogenicity are obligatory studies for the preclinical assessment of drug safety; current methodologies require the sacrifice of a large number of animals and complex biologic tests to measure these toxic effects. We have generated a novel, simple, quantitative and robust assay based on noninvasive imaging technologies to measure in vivo the state of cell proliferation in any body tissue. The assay is performed in the repTOP*mito*IRE, a genetically modified mouse which carries a luciferase reporter gene under the transcriptional control of a minimal human cyclin B2 promoter. We have extensively demonstrated in validation experiments that in the repTOP*mito*IRE, the photon emissions from the tissues are proportional to the rate of cell proliferation. The methodology we have developed allows: i) a direct measurement of the toxic effect of the drug under study on the proliferation of bone marrow progenitors (myelotoxicity) or ii) the quantitative detection of undesired proliferation produced by a treatment in any body tissues (carcinogencity). We here provide explanatory examples of the application of this assay to the measurement of the myelosuppressive effects of chemoterapic agents (5-fluorouracil, docetaxel, bortezomid, temozolomide) and of radiotherapic treatments. The potential of the carcinogenicity test was demonstrated with the administration of well known carcinogens such as dimethylbenzantracen and phorbol esters. The possibility to follow in time, in the same animal these effects greatly exploits the information produced as well as the statistical power of the analysis, while reducing costs and number of animals used in the assay.

P261 Lung Ultrasound in the Ovine Experimental Model of Pneumothorax and Pleural Effusion

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Lung ultrasound (US) is raising popularity in the clinical emergency settings. It's more sensitive than chest-radiography for pneumothorax and pleural-effusion diagnosis, showing a good correlation with gold standard CT. Teaching the complexity of lung US might be difficult in unstable patients; therefore experimental animal models for teaching lung US examination are needed. Herein we describe our experience with an ovine model for US lung diagnosis of pneumothorax and pleural effusion. Under general anesthesia, and with animal in dorsal recumbency, US assessment with 4 Mhz convex probe of the normal lung was performed in 2 sheep. A thoracic drain was introduced in right pleural cavity. US was repeated after air was introduced in the pleural cavity. Subsequently air was drained and saline was injected into pleural cavity. US detection of pleural effusion was made. All measures were performed by two examinators. Dynamic and static US signs of normal lung were successfully detected: lung sliding, lung pulse, A-lines, and the seashore-sign, all originating from the pleural interface. Detected US pneumothorax signs were: lung point, stratosphere sign, absence

of lung sliding and of B-Lines. The ovine model is suitable to lung US assessment, exhibiting identical signs to the ones found in the clinical settings. As sheep is widely used on thoracic surgery research, not only medical practitioners but also veterinarians could gain advantage training this technique. In the experimental surgery unit, lung US can provide a new noninvasive refinement tool in the postsurgical assessment of sheep that underwent thoracic surgery.

P262 Effect of Repeated Bleeding on Blood Glucose in Rats: A Modified Tail-Vein Cannulation Compared to Tail Venepuncture

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Repeated blood sampling from tail vein is extensively used in pharmacokinetic and pharmacodynamic protocols. Choice of the method ensuring the minimal stress to animals avoids bias to the final data and is mandatory for the 3 R's. The aim of present study is to compare the impact of two tail blood sampling techniques on blood glucose level in rats as a parameter of stress. Fourteen male Wistar rats were divided in two groups and subjected to vein cannulation (group A) and venepuncture (group B).0.5 ml of blood was collected at 0, 30, 60, 120 and 240 minutes after surgery in group A and, at the same time points, in group B. Since implanted cannula are susceptible to rat bites, a special device protected by a light stainless steel net was used. This refinement allows us to maintain cannulated rats in freely moving conditions as in group B. At time point 0, group A showed statistically significant ($P < 0.00$) greater level of glucose compared to venepuncture bled rats. Moreover, glicemia detected in the following time points did not differ statistically between groups, indicating a comparable stress level in the experimental animals. The tail-vein cannulation provides a series of advantages over tail venepuncture: warming of the animal is not necessary; the cannula ensures rapid blood withdrawal at all time points; it is possible to obtain large blood volumes in a short time and to replace them with saline. In conclusion our refined tail method appears to be an appropriate alternative to venepuncture.

P263 Improved Study Planning and Working Processes to Reduce the Number of Mice Used in Research Projects

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By investigating selected standard experiments and using more sophisticated models like humanized mice and developing new processes for generation of study cohorts we have found it possible to reduce the number of required animals while saving valuable time and funds. The development of sophisticated genetically modified mouse and rat models has aided biomedical research immensely and refined the methods while reducing the number of animals used. For instance, by using a humanized mouse model researchers have reduced the number of animals required in standard carcinogenicity testing from 400 animals to 50 animals, while also reducing the required study time from 2 years to 26 weeks. Several genetic models exists which utilized in oncology research reduces the number of animals used 4-5 fold and give much more precise answers than more traditional models. By humanizing the mice or rats; either by tissue humanization or by genetic humanization, the scientists have access to more predictable models which by refinements of methods reduces the required study cohort size. An optimal planning and design of the genetic modification of the embryonic stem cells combined with a successful injection in blastocysts and the transfer into recipients with high reproduction performance and defined flora reduces the time for the study cohort production. New processes integrating genetic model creation and expansion breeding are reducing the number of animals required to produce a sufficient sized study cohort. With a targeted approach the size of the study cohort can be further reduced compared to using traditional animals.

P264 Sciatic and Femoral Ultrasound-Guided Nerve Block in the Ovine Model of Critical Size Bone Defect

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The sheep is commonly used as a preclinical animal model in orthopedic research. Experimental procedures as critical size bone defects involve severe postoperative pain, which should be approached with a multimodal analgesia regimen, combining parenteral pain-relief drugs with regional anesthesia. The main objective of this study was to describe the ultrasound-guided nerve block technique for sciatic and femoral nerves in sheep. The secondary objective was to provide a minimal motor block of the limb nevertheless maintaining sensitive blockage. Prior to the in vivo study, ovine cadavers were used in order to examine the anatomic references and nerve localization. Three 2-years-old Ripollesa breed sheep underwent ultrasound-guided nerve block. Nerve visualization was achieved using a 2-5 MHz convex ultrasound transducer, taking as reference bony and vascular structures. Nerve stimulation was used to assist in verifying position of the needle tip in relation to nerve structures. Target was a distal twitch (sciatic nerve) or thigh twitch (femoral nerve) at 0.5 mA stimuli. Ropivacaine 0.2% 1 mL was injected to surround the nerves completely. The efficacy of the nerve block was assessed concerning bearing ability and sensitive function through noxious stimuli every thirty minutes. Sheep showed a smooth recovery from general anesthesia, exhibiting no motor block whereas the average duration of the sensitive blockage was 340 (SD 17.32) minutes. Ultrasound guidance for nerve localization provides anatomic detail and accurate placement of local anesthetic in real-time thus ensuring a successful nerve block which allows better pain control and therefore better welfare in experimental animals.

P265 Refining the Rabbit Experimental Model in Orthopedic Research: The Use of Ultrasound-Guided Technique in the Axillary Brachial Plexus Nerve Block

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The rabbit is one of the most commonly used animal models in orthopedic research. Refining analgesia procedures in this model is imperative, as severe postoperative pain is usually associated to orthopedic surgery. In addition, cellular-therapy trials recommend not using NSAIDs to avoid interference with stem-cells performance, reducing the drug spectrum options for post operatory pain-relief. The aim of our study is to define the usefulness of ultrasound-guided regional anesthesia in the rabbit experimental model in the orthopedic research. Ten 9-12 weeks-old Whyte New Zealand rabbits underwent ultrasound-guided nerve block of the axillary brachial plexus, under general anesthesia. We used a 12MHz lineal transducer, 35mm neurostimulation needle and aimed for a forelimb twitch at 0.5 mA stimuli,

infiltrating 0.9 to 1.2 mL of ropivacaine 0.3% around the nerves. Ultrasonographic guidance allowed visualization of anatomic structures, neurostimulation needle and real-time anesthetic distribution. Effective nerve block was assessed by monitoring pinch withdrawal every thirty minutes in every rabbit. Average time needed to perform the nerve block was 4.5 (SD 0.85) minutes and the duration of sensitive blockage were 360 (SD 49) minutes. No adverse reactions were observed. To our knowledge, this is the first description of the echo-guided technique for nerve block of axillary brachial plexus on the rabbit. We concluded that this ultrasound-guided technique is feasible, reproducible, safe and not time-consuming. It provides good analgesia of the forelimb, and can be used routinely as an important refinement tool integrating multimodal analgesia approach. This refined technique allows the improvement of rabbit welfare after forelimb orthopedic surgery.

P266 Comparative study of Moxifloxacin Pharmacokinetics in Rat: Accusampler System Compared with Manual Sampling

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The AccuSampler is a fully automated system for automatic blood sampling and injection of compounds in freely moving animals. The animal is connected to the AccuSampler through a surgically implanted catheter leading to a stainless steel swivel mounted on a balanced lever arm. All steps in the sampling procedures are monitored and logged by the AccuSampler Software. An antitubercular drug (Moxifloxacin) was used for a preliminary pharmacokinetic characterization using Accusampler and compared with manual sampling. Male Sprague–Dawley rats were cannulated in both left and right jugulars and split into two groups (n = 3/group), samples were obtained from the right catheter either manually (Manual group) or with the Accusampler (Accusampler group). The compound was assayed in single intravenous dose through left catheter at 3 mg/Kg. Additionally, in order to evaluate whether samples needed to be collected immediately after sampling, an aliquot of each blood sample was mixed with saponin immediately, while another aliquot was left overnight refrigerated in the Accusampler rack until the next day. We found no significant differences in pharmacokinetic profiles and pharmacokinetic parameters between Manual and Accusampler groups, neither between different stations of Accusampler. No significant differences were found between aliquots collected just after sampling or the next day. In conclusion, samples obtained with the Accusampler are comparable to those obtained manually through a catheter. In addition, using automatic blood sampling systems, animals are undisturbed and do not experience stress during the sampling procedure, sampling at late hours is effortless, and less staff is required compared to manual sampling.

P267 Refining Handling Methods in Mice

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A novel method of handling mice with a tunnel has been shown to cause less aversion and anxiety than tail handling. Our aim was to test whether tunnel-handled mice continued to show reduced aversion when subjected to a standard experimental procedure (single dose pharmacokinetic study). Twenty-two female C57BL/6J were split into pairs, and handled for 30 seconds twice a day, either by the tail (controls) or with a tunnel, Monday to Friday for 8 handling days. Aversion to the handling procedure was determined by measuring time of voluntary interaction with the hand/hand+tunnel for one minute before and after handling on the first day of handling (S1), the day before the PK (S8) and the day after the PK (S9). During the PK study, behavioral reaction to handling was also scored. Time of voluntary interaction was always higher for tunnel-handled mice, although only significantly in S8, maybe indicating a general reduced aversion to the tunnel. Time of voluntary interaction significantly increased in S8 when compared to S1, showing habituation to both methods. There were no significant differences in behavioral score during the PK study. However, time of voluntary interaction after the PK study (S9) was significantly higher than in S1 for tunnel-handled mice but not for controls. This probably indicates that handling during the experimental procedure was less stressful for tunnel-handled mice. Tunnel handling is a time-consuming but effective method of reducing aversion associated with handling mice during experimental procedures

P268 Systematic Reviews and the 3Rs

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Although the implementation of 3R methodologies in research is obligatory in most countries, the search for relevant 3R methods and eventually their implementation can be very challenging. Survey results indicate that it is not uncommon that existing 3R possibilities are neither found nor implemented. This may be explained by the fact that 3R information is scattered over various sources (for example, various databases and textbooks). Here the first steps of the Systematic Reviews (SR) methodology may be a part of the solution. When planning and designing a new animal experiment a number of questions need to be answered, for example, What evidence is already available? Is an animal experiment necessary and, if so, which animal model is most suitable? These questions can be answered by conducting a thorough literature search. The main advantage of doing a systematic search is in this stage is that it provides more structure and transparency. Transparency of the search process is needed for reproducibility of the search. Additionally, it can help Animal Welfare Officers and members of Animal Ethics Committees in their evaluation of the design of new animal experiments. SR steps, such as systematic search, but also the risk of bias assessment of primary studies, will provide important insights necessary for the evidence-based design of a new animal experiment. Moreover, a structured and documented systematic search of literature as the first step in performing systematic reviews, will also meet the new requirements according to the EU Directive 2010/63.

P269 SYRCLE: Systematic Review Center for Laboratory animal Experimentation

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SYRCLE is a unique and innovative research and educational center within the Central Animal Laboratory of the Radboud University Nijmegen Medical Center. The aim of SYRCLE is to continuously improve the scientific quality and transparency of laboratory animal science, including animal welfare. To achieve this goal, SYRCLE has adopted Systematic Reviews (SR) as a methodology for Synthesis of Evidence (SE) in animal studies. In 2012 SYRCLE organized the first international symposium on Systematic Reviews in Laboratory Animal Science. SYRCLE's research activities encompass the development of tools (for example, search filters) and guidelines. The Dutch Ministry of Health has provided funding to support the development of methodology and tools. SYRCLE also assesses the methodological quality of recently conducted SRs of animal studies. The Dutch funding agency ZonMW has commissioned a special educational program at SYRCLE for 'hands-on training' workshops in the Netherlands. The full educational program of SYRCLE ranges from introductory lectures on the methodology of SR to complete 'train the trainer' programs. SYRCLE currently cooperates with various clinical departments in writing SRs of animal studies. SRs of animal studies are used to attain an evidence-based choice of animal models, improve the design of new clinical trials, and clarify differences

between the design of animal studies and clinical trials. The primary focus is scientific communication via open access publication of SRs and other related research outcomes. Additionally, SYRCLE communicates (inter)national news items on SE and SR of animal studies by using social media, newsletters and at the website www.syrcle.nl.

P270 The Ethical Comittee and the 3Rs Principles

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Despite a real wish of the scientific community to use as much as possible in vitro models, Biologic, Medical and Pharmaceutical research still relies heavily on animal testing. Today the progress in veterinary science has allowed us to better understand all factors influencing animal welfare and the impact it had on the quality of experimental results. Therefore it had considerably changed conditions use of animals for experimental purposes. This shift took place through the gradual establishment of ethics committees in all research institutes and with the application of the 3Rs. On the principle of 3R to Replace animals, to Reduce the number of animal used and to improve the experimental conditions (Refinement), a real willingness to work together exists between researchers, technicians and ethics committee members realizing that ethics is not only a regulatory requirement but that more each actor has something to gain with a collaborative approach that contribute to its success. Twelve years after the creation of the first ethical committee at the site, the ethical approach is supported by many actors. Thus proposals in relation to the 3Rs come from several sources: scientists who propose to integrate new techniques to be tested in their studies (for example, enrichment), operationals implementing new techniques to reduce the number of animals induced stress (for example, DBS). Thanks to this collective involvement, a real partnership is developed among teams and gradually the ethics committee and the newly animal welfare unit set up under the new Directive 2010/63/UE strengthen their advisory role.

P271 Is Ketamine Alone or Combined with Midazolam or Dexmedetomidine a Good Anaesthetic to Use in Biomedical Research that Requires Behavioral Evaluation?

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Most biomedical experimental procedures on animals require analgesia or/and anesthesia to avoid/ reduce stress and suffering. However, anaesthetics may influence animal behavior and interfere with the experimental results. Hence our goal is to study the effects of ketamine alone or combined with midazolam or dexmedetomidine on spatial and recognition memory in rats. Male Wistar rats were randomly distributed into four groups: saline control, 100 mg/kg ketamine, 100 $mg.kg^{-1}$ /5 $mg.kg^{-1}$ ketamine/midazolam, and 100 $mg.kg^{-1}$ /0.25 $mg.kg^{-1}$ ketamine/dexmedetomidine; each received one intraperitoneal injection. A radial maze test (RAM), and a novel object recognition and location test (NOR/L) were performed 48 hours post-anesthesia with different batches of rats. Regarding the number of working and reference memory errors in the RAM test, no differences were found between groups. In the NOR/L test, all the tested subjects recognized novelty, as shown by increased exploration of the new object and the object in the new location when compared with the familiar condition. When facing the object for the first time in the NOR/L initial session, control rats approached it more often than the rats from the ketamine/midazolam and ketamine/dexmedetomidine groups. Nevertheless, this difference was no longer observed two minutes afterwards, indicating that all animals rapidly adapted to the new environment. In conclusion, a single administration of ketamine alone, or combined with midazolam or dexmedetomidine can be safely used in adult rats regarding memory evaluation. Overall, these results contribute to the enhancement of animal welfare in neurobehavioral procedures without interfering with the quality of the results.

P272 Reduction of Animal Numbers in Pharmacokinetic Studies: A Retrospective Analysis

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In the early phases of drug discovery research, pharmaceutical companies have to evaluate the biologic properties of large numbers of biologically active compounds. This biologic characterization demands, among others, preliminary pharmacokinetic (PK) and toxicology studies at early stages of drug development. At this level, the mouse is the specie of choice both for practical and scientific reasons. However, preliminary PK studies in mice usually involve large numbers of animals that are euthanized for blood collection at each time point of the study. In FELASA 2007 we presented a study that aimed at reducing the amount of animals used in this kind of studies by moving from population pharmacokinetics to serial sampling in individual mice. In the following years, we have reduced population PKs from 80% of the total PKs in 2009 to less than 1% in 2012. As a consequence, animal numbers have been reduced in an 85%, even when PK studies have increased by a 34%. Additionally, serial PKs have been refined by reducing blood sample volume from 25 µL per time point to 15 µL. This has been possible thanks to the high sensibility of liquid chromatography coupled with tandem mass spectrometry (LC/MS/MS) -which requires minimal samples for compound determination- together with the dexterity of our animal technicians. This retrospective analysis shows how a dramatic reduction in animal numbers is possible with a commitment to the application of the 3Rs principle and through the use of new technologies, allowing an improvement in animal welfare while maintaining high-quality science.

P273 Animal Welfare Consideration for In Vivo Imaging of Laboratory Rodents

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The use of laboratory animal imaging has become an instrumental tool in biomedical research, enabling a powerful, noninvasive and clinically translatable way for monitoring disease progressions in real time and testing new therapies. Their capability for providing detailed in vivo anatomic and functional data in live animals is one of the key advantages. In contrast to human studies, however, imaging of animals generally requires anesthesia, and anaesthetic agents have a profound effect on the animal's physiology and may thereby confound the image data acquired. In addition repeated anesthesia, exposure to ionizing radiation, administration of contrast agents, environmental consideration and animal biosafety issues related to the imaging suites may affect the processes under study. We will discuss how these challenges can be successfully addressed through an appropriate understanding of the most commonly used preclinical imaging technologies, including computed tomography (CT), magnetic resonance imaging (MRI), positron emission tomography (PET) and single photon emission computed tomography (SPECT) and optical based technologies. We will review the protocols for animal preparation, anaesthetic regimes and current techniques for physiologic monitoring, based on our experiences imaging a broad variety of preclinical models. Issues related to animals transfer and biosecurity, multidisciplinary imaging and repetitive acquisitions for longitudinal studies will also be addressed. Imaging can make a substantial contribution to the 3Rs while providing more informative and

humane readouts in living animals. But it is important that procedures are continuously refined to minimise impact on the studied biologic processes while ensuring animal welfare.

P274 Blood Glucose Concentration in Rats, Is More Affected by Anesthesia with Ketamine than Fentanyl

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It is known that anesthetic and sedatives agents can alter the plasma concentration of some biochemical parameters. Glucose is one of the most frequently biochemical parameters determined in multitude of rodent experimental procedures. Some of the most common parenteral anesthetics used in rats, such as Ketamine and Fentanile, induce hyperglycemia, which can be explained in vitro, at least partly, by impaired glucose-induced insulin release. Furthermore medetomidine, a sedative frequently used, inhibit (in vitro) insulin secretion through an α(2)-adrenoceptor. So, the aim of this study was to know what magnitude it would be affected by the anesthetic protocol that we are using. For this, we anesthetized 20 male Sprague–Dawley fed rats of 10 weeks. They were separated in two groups: Group A ($n = 10$) was anesthetized ip with Ketamine/Medetomidine (100/0.25 mg/kg) and Group B ($n = 10$) was anesthetized ip with Fentanyl/Medetomidine (0.3/0.3 mg/kg). Each rat was performed two determinations of blood glucose (before anesthesia and 15 minutes after anesthesia). The measurement was made by a drop of blood from the ventral tail vein and it was analyzed with a quick lecture glucose-test system. The analysis of results showed statistically significant differences in pre-and post-anesthetic blood glucose with both protocols. But if we compare the post anesthetic glycemia in both protocols, we note that there is a greater increase in Group A (274.8 + 39.8 mg/mL) than in Group B (223.7 + 26.04 mg/ml) P overall (0.006).

P275 Hematological Parameters Vary Depending of the Place of Blood Sampling in Sprague–Dawley rats

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Numerous blood collecting methods have been described for the rat, and the bleeding site and anaesthetic agent have been shown to have an impact on hematological parameters. Unfortunately, in all of these comparative studies, some of the puncture sites and/or anesthetics protocols were different to we used. In this study, we compared the erythrocytes, leukocytes, and hemoglobin concentrations from blood samples collected terminally from the right Brachiocephalic vein (BV), left Jugular vein (JV), Aorta (A) and heart (H) in order to determine whether the sampling method has an influence on clinical pathology parameters. Ten 10-week-old male Sprague–Dawley fed rats were anaesthetized with fentanyl/medetomidine prior to sampling. For sampling from the BV, they were punctured below the clavicle. For sampling from the YV, it was exposed. From the A, the abdominal A was exposed and from the H, they were punctured through the left chest wall. Erytrocites quantities observed indicated differences ($P < 0.05$) between A (6.93 + 0.32×10^{12} /L) compared with JV (7.29 + 0.38×10^{12} /L), H (6.82 + 0.32×10^{12} /L) compared with JV and BV (7.17+0.32×10^{12} /L) compared with H. Hemoglobin results were BV (14.61 + 0.43 g/dL), JV (14.86 + 0.45 g/dL), A (14.22 + 0.30 g/dL), and H (13.92 + 0.37 g/dL) with significant differences ($P < 0.05$):H compared with BV, H compared with JV, and A compared with JV. Leukocyte quantities observed indicated significant differences ($P < 0.05$) between A (6.35 + 2.03 × 109/L) compared with BV (8.39 + 2.09 × 109/L), A compared with JV (10.18 + 2.12 × 109/L) and H (7.00 + 1.97 × 109/L) compared with JV.

P276 Cryopreservation of Mouse Ovaries: A Method for Banking Supporting the 3Rs Rule

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In many facilities, genetically modified mouse strains are maintained in a cryopreserved state providing a 'back-up' against the loss of mouse lines due to breeding errors, genetic drift, or genetic contamination. The first banking technology was embryo cryopreservation which required a large number of donor animals. Sperm freezing is now commonly used since it requires only a few donor males; however, a large number of females is also needed for in vitro fertilization at the time of revival. A third technique, cryopreservation of mouse ovaries, has been more recently described. Ovary cryopreservation only requires few females. Revival is based on orthotopic transplantation of cryopreserved ovaries (Sztein et al,1998). Nine transgenic facilities from the French ROCAD network have tested this technology for freezing and revival of mouse lines from various pure or mixed genetic backgrounds. Different published protocols have been tested to define the best results for banking and revival. Our results show that one female donor can reconstitute four fertile recipient females. We have been able to resuscitate all tested strains, using never more than four grafted females. We observed a significant but small difference between lines in the delay to obtain progeny; however, progeny have been obtained in all cases. Compared to other banking techniques, ovary cryopreservation is robust, and requires few animals for freezing and also revival. The technique is easy to use for transgenic facilities where embryo transfer is a routine. For technical and ethical reasons, the use of this technique should be encouraged.

P277 Percutaneous Vascular Access Techniques in Preclinical Animal Models: The Ultrasound-Guided Vascular Access as an Important Refinement of the Blind Technique

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Preclinical animal trials require frequently the access to femoral vasculature and the advance of large catheters, traditionally placed by surgical dissection. Direct percutaneous puncture using Seldinger technique increased popularity. Ultrasound (US) guidance for percutaneous vascular access is commonly used in the clinical settings. The aim of this study was to compare complications associated with percutaneous vasculature access, using blind compared with US-guided approaches to femoral deep vasculature in the species used commonly as preclinical models in our lab (pigs and sheep). Criterion for study-inclusion was animals that underwent femoral vascular access, independently the surgical procedure that was performed after. Animals included were

18 minipigs, 10 pigs and 5 sheep. Times needed for vascular access establishment, intra/postoperatory complications in puncture site or related with the procedure were recorded and compared. Arterial access required an average of 56 (SD 36) minutes in the blind-technique and 7 (SD 3) minutes in US-guided access. Venous access required 21 (SD 15) minutes in the blind-technique and 5 (SD 2) minutes for the US-guided approach. Blind-technique had 10% unsuccess on arterial access, requiring posterior surgical dissection. US-technique had a 100% success in arterial/ venous access establishment. Reutilization of the vessels was possible in both percutaneous techniques, in contrast with dissection. Postsurgical infection in access site was detected only on dissection. One animal died after blind-procedure, due to undecteded arterial hemorrhage. The implementation of US-guided femoral vascular access as a routinely approach in the lab contribute to refine the animal models, being less time-consuming and reducing procedure-related complications.

P278 Nonsurgical Embryo Transfer with the NSET Device is a 3Rs Refinement Technique that Reduces Stress in CD-1 Mice

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The Transfer device (NSET™) from ParaTechs is a nonsurgical method for embryo transfer in mice. The NSET device is a Teflon catheter that attaches to a P2 pipette and enables the transcervical transfer of embryos into the uterine horn. The NSET technique can be used to transfer blastocysts during the generation of transgenic mice, after cryopreservation, after in vitro fertilization, and during rederivation of mouse strains. We hypothesized that the NSET procedure is less stressful than surgery for recipient females, but this claim had not previously been investigated. In this study, we examined the stress response of recipient mice undergoing either the NSET procedure or surgery. Stress was quantified by examining the levels of fecal corticosterone (a stress biomarker) and through electrocardiograms that identified changes in heart rate and cardiac rhythm variability in response to each procedure. ELISA analysis revealed a 2.5-fold increase in fecal corticosterone levels 3 to 10 hours after surgery. This effect was not seen after the NSET procedure. Electrocardiograms showed a 50% decrease in heart rate as a result of anesthesia, and an increase in heart rate variability after surgery. The NSET procedure elicited no significant changes in cardiac output or rhythm. These results demonstrate that the NSET device is a refinement as defined by Russell and Burch's "3Rs" and should be considered as a replacement for uterine embryo transfer surgery whenever possible.

P279 New Replacement Alternatives Used for Training Students in Veterinary Medicine in the Netherlands

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Over the last 2½ years the Faculty of Veterinary Medicine, Utrecht University, The Netherlands and the Dutch Society for Replacement of Animal Testing (DsRAT, Proefdiervrij) have discussed possibilities to further reduce the use of laboratory animals for teaching and training veterinary students. The deliberations have led to the signing of two formal agreements between both parties. Until recently animals, mainly dogs and cats, that were frozen or formaldehyde fixed after euthanasia upon arrival were used for teaching knowledge of anatomy in practical classes at the Faculty of Veterinary Medicine in Utrecht. A similar approach was used for practical classes in which students were trained surgical skills. The Utrecht University and DsRAT agreed to join forces to introduce a body donation program aiming at a full replacement of laboratory animals by pets euthanized for health related causes. After roughly half a year the initiative already can be called a success. DsRAT has also agreed to substantially support the Utrecht University to further develop plastinated models of animals to replace the need for carcasses. This assistance allows for the production of a number of educational boxes containing a variety of detailed body parts of different animal species for teaching and training veterinary anatomy. Both projects are a typical demonstration that parties that may have conflicting points of view can nevertheless find ways to join efforts so as to reach goals of mutual interest.

P280 Dorsal Subcutaneous Injection Site Effect on Vaccines Potency Tests

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In the context of human vaccines batch release, international or national legislations require in vivo potency studies. Most of these tests are based on the evaluation of the seroconversion of the animals after a single immunization. The outcome of the study is often a single value reflecting the amount of specific antibodies produced (or a relative value when compared to a reference vaccine). Mice subcutaneous injections are often the primary choice for those assays. GMP studies comparing two dorsal subcutaneous injection methods (anterior or posterior localization, using 22-gauge needle) on mice (NMRI, BALB/c, ICR) are set-up in 4 different in vivo vaccines potency models. After the immunization period (depending of the model), sera are analyzed to calculate the potency value. Statistics are used for sample size and final analysis to evaluate and quantify differences (IC 95%). All the parameters (sample, dilution, housing…) are fixed to guarantee that the only variable is the inoculation method. This poster reports the significant impact of small changes in the localization of the subcutaneous injection and highlights the importance of clear description of those parameters in method validation processes. Accurate Standard Operating Procedure and efficient training program are the key elements to include in an initial validation of an in vivo potency test in order to guarantee an optimal reproducibility of the studies and a good application of the 3Rs principle. Benefits of this approach are the simplification of method transfer between sites and release authorities, Reduction (3Rs) and the increased scientific power of the assay.

P281 Learning Ability Test for Juvenile Cynomolgus Monkeys: Do the Results Justify the Effort?

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Learning ability is an endpoint in juvenile chronic toxicity studies and sometimes requested by regulatory authorities, especially if the examined drug targets the nervous system. A double choice object discrimination task using a Wisconsin General Testing Apparatus (WGTA) was performed on 56 juvenile cynomolgus monkeys (28 animals/gender, aged 9 to 10 months) to test for learning ability in the predose phase of a general toxicity study. At first a habituation phase was performed to acclimate the animals to the situation and to train them to remove objects (cubes) for a food reward. Afterwards a test was performed to detect a possible preference for one of the objects (cone or hemisphere) of the learning phase. The nonpreferred object was then used as rewarded object in the learning phase. A daily session contained 20 trials; the learning criterion was 80% correct choices. Once the learning criterion was reached on two consecutive days, the definitive learning test was performed. Juvenile males allocated to the study passed the learning test on average after 9.5 days, while juvenile females needed on average 13.6 days to complete the task. Power analysis showed that with an $\alpha = 0.05$ and an one-sided t test, a sample size of 55 for the males and 47 for the females would be necessary to predict a 50% change of learning duration, and even for an 100% change a group size of 13 to 15 animals would be necessary. However, results of learning ability gained during the study still can be compared to individual predose data.

P282 Case Report: Sonography in Vaginal Septum Diagnosis

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Vaginal septum is a congenital malformation, which can affect up to 11% of the females in a C57/BL6 mice colony, and might cause a decrease in the colony fertility of about 75%. Reported a case of vaginal septum diagnosed with a sonovaginography. The animal was anesthetized with an oxygen/isoflurane rate of 4% during the induction and 2,5% during maintenance. The mice got its perineal area shaved and the vagina was filled with ultrasonographic jelly with a syringe and a plastic cannula, in order to increase its ultrasonographic contrast with the surrounding tissues. The exam started with an echolocation of the vaginal cavity. The cavity was checked, looking for a disruption of the anecoic cavity. An exam of the reproductive tract was performed, in order to analyze the possibility of a uterine duplication, an associated process to vaginal septum. We confirmed the vaginal septum, as a hipoechoic structure that divided the vaginal cavity. Uterine neck and horns were completely normal, as were the ovaries. A vaginal septum without uterine affection was diagnosed. The experiment describes a more accurate method to assess the extension of the septum, including uterine body and horns, which can be carried out in a short period of time and with a small cost. Localizing the pathologic animals could mean an increase in the fertility of the mice colony, translated to a reduction in the number of animals and the economic cost of the animal facility.

P283 Withdrawn

P284 Creation and Management of an Ethics and Laboratory Animal Welfare Committee in Chile, the Reality, Achievements and Challenges

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In 2005, considering the requirements of Scientific Journals regarding ethic/bioethics regulations, the growing development of scientific research with living beings and to strengthen scientific activities, the main chilean government research funding source, CONICYT, decided to create an Advisory Committee in Bioethics. That event should have promoted the creation of Bioethics Committees in all research institutions in our country. However, this has been a very slow process. In Chile, there are few professionals trained in Ethics, Animal Welfare and Law to set up regulations and standardize procedures at national level; therefore, it is each institution's responsibility to develop such regulations. Despite the recent law regarding Animal Protection, there is no national consensus on the operation of bioethics committees, animal maintenance conditions, standard operating procedures, etc. It is unknown how many IACUCs do exist and how do they work, who are the people behind protocol reviews, in what conditions are animals maintained, which guidelines do they approve and ascribe, what kind or training, if any, do researchers receive, etc. Here we show how we applied innovation tools to create a functional and efficient committee, what achievements we have reached and what challenges we are still faced with. We understood the importance of the 3Rs. We educated our scientific community. We defined certain regulations and standardize procedures according to our Institution's reality. As a nation, our mail challenge is to set up qualified Committees nationwide that are able to introduce regulations in each Research Institution in order to aim for international standards.

P285 Assessment of Chronic Stress in Nonhuman Primates by Determination of Hair Cortisol Levels

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Short-term changes in glucocorticoid levels are relatively easily assessed in biologic fluids, such as serum and urine. Values in these fluids reflect acute to subacute values and can be highly variable. Recently, methods have been developed to determine cortisol levels in hair. Cortisol in the circulation is incorporated into the hair shaft during growing and the amount reflects the average levels over the growth period of the hair, making it suited to determine chronic hormone levels. We determined cortisol levels in hair samples of 150 long-tailed macaques before and after their move from their original location to the BPRC with a follow-up of more than 2 years. In addition, we sampled our colonies of rhesus macaques and common marmosets. The results show that hair cortisol levels increased in long-tailed macaques due to moving followed by a gradual decline thereafter. In the new housing situation, cortisol levels were significantly lower than those measured in their original location. Rhesus macaques and long-tailed macaques show comparable cortisol levels in hair samples at the BPRC, but levels in marmosets were much higher. We also observed age-related differences in all three species. We conclude that hair samples of nonhuman primates can be used to determine cortisol levels reflecting prolonged stress levels. This method can be used as a minimal invasive technique to monitor welfare of these animals in captive and/or experimental conditions.

P286 Laboratory Animal Use in Chile: Current Situation and Issues

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Collaboration between developed and developing countries in biomedical research involving laboratory animals has increased considerably in the last decade. This brings about issues related to both animal care and use standards, and differences in legislation between these countries. Besides, it is well documented that different environments elicit different responses in rodents of the same genetic strain. Thus, we consider it is essential to characterise vivaria and to try to homologate housing conditions and animal care standards. In Chile, there are no official statistics about animal use. As a reference to its recent evolution, we know that the main national laboratory animal provider steadily increased its production from 67,000 to 195,000 mice between 2000 and 2011. We also know that there are between 15 and 20 vivaria, established as such. Contrast is high: only one vivarium is AAALAC-accredited, while in some places it is still possible to have a small colony, housed in an academic's lab, in conditions that are neither controlled nor supervised by an IACUC. Fortunately, this is now a minority situation. Most institutions are making efforts to set higher standards both regarding health and animal welfare. In addition, several organizations, mainly in academia, are setting up bioethics committees in order to review research projects addressing unnecessary animal suffering by implementing the 3Rs. We will present the results of an ongoing national survey of rodent vivaria and their characterisation.

P287 Animal Research in a Global Organisation: Advantages and Challenges of a Centralized Oversight Group

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Regulations governing animal care and use vary throughout the world, the laws reflecting the differing cultures of regions and countries. This presents international organisations with a challenge if the wish all of their research to be carried out to a common standard. Our

company addresses this by having an overarching policy on animal care and use which lays out a set of core principles that must be met for all animal studies irrespective of their location or whether they are carried out within company facilities or performed externally on our behalf. In 2012, we established a centralized group, independent of the operational responsibilities (husbandry, care, study design and support), to lead the company vision for responsible animal research. This group, the Office of Animal Welfare, Ethics and Strategy (OAWES), is responsible for identifying strategy, supporting global initiatives and training, as well as providing governance and advocacy in the animal research and 3Rs space. This presentation describes the advantages and efficiencies which have resulted from formation of OAWES and outlines the sucesses to date along with the challenges we have faced during our first year of operation.

P288 Superovulatory Response and Preimplantation Embryo Development In Vitro in 2 Senescence-Accelerated Mouse (SAM) Strains: SAMP8 and SAMR1

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A series of inbred strains of mice have been developed that are either prone (SAMP) or resistant (SAMR) to accelerated senescence. These strains originated from an inadvertent cross or crosses between the AKR/J strain and an unknown strain(s). The characteristic feature of aging common to the SAMP and SAMR is accelerated senescence and normal aging, respectively. Both strains are widely used in aging studies; however, their reproductive characteristics are not well known. In this study, we have assessed the superovulatory response and the in vitro embryo development in 6- and 10-week old females from SAMP8 and SAMR1 strains. C57BL/6J mice were used as control. Females were treated with 7.5 IU equine chorionic gonadotropin (eCG) followed by an injection of 5 IU human chorionic gonadotropin (hCG) 48 hours apart. In a group of females of each age (n = 11), oocytes were collected 20h post hCG. Another group of females (n = 5) was mated to males and zygotes were collected 20 h post hCG and cultured in vitro at 37 ºC, 5% CO_2 in M16 medium. The results show that the numbers of oocytes were greater in SAMR1 than in SAMP8 mice independently of the age of the females. In SAMR1, the superovulatory response was better in 10-week old mice compared to 6-week old females. We observed that most of the embryos (about 95%) from SAMP8 and SAMR1 females were blocked at 2-cell stage. We think these results can be very useful to perform assisted reproductive techniques in these strains.

P289 Effect of Light Intensity on the Exploratory Behavior of C-57 Mice

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There is widespread agreement on the need for standardization of parameters that might affect behavioral data generated in phenotyping experiments in different laboratories in order to minimize the variability of results and waste of animals. It is generally accepted that light could play an important role on animal welfare in general and on the outcome of exploratory tests, especially in nocturnal animals such as mice or rats. Surprisingly, the specific light intensity being used in the experiments described in the bibliography is usually missed or poorly defined. In this study we sought to address the differences in the exploratory behavior of C-57 females at three light intensities (40,250,600 lx) in control conditions and after injection of diazepam or epinephrine. Results show similar distance travelled over the 30 minute assay of our open field test but a marked increase in anxiety related behavior at 600 lux compared to 40 or 250 (but not between 40 and 250 lx). Treatment with any drug outweighed the effect of light, however, the significant differences in specific parameters found between control and treated animals varied depending on the light intensity. Differences in permanence time in the periphery of the arena were best seen under dimmer light while divergence between drug exploration was higher at medium intensity. Our results seem to indicate that illumination of the arena should be taken into careful consideration when conducting exploratory analysis of mice, but the optimal intensity in which the animals must be placed may depend on the specific analysis being conduced

P290 Comparative Study of Wound Healing in New Zealand Rabbit Using Adhesive and Suture

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There are a few scientific studies dealing with the properties of wound healing using classic suture and the new cyanoacrylate adhesives. In this study, we compare those different methods, paying particular attention to their performance for in vivo studies carried out with New Zealand white rabbits. We use the same animal for three different experiments in order to apply 3Rs rules, so we refine the methods and reduce the number of animal. Some physico-chemical characterization of adhesives is very important in the effectiveness of the tissue repairs. In general, the temperature of decomposition increases by increasing the length of the alkyl chain in the cyanoacrylate. The butyl cyanoacrylate (BCN) adhesive is very aggressive on the rabbit skin due to high exothermic reaction. Both wound closures obtained with ethyl cyanoacrylate (ECN) and octyl cyanoacrylate (OCN) are quite similar. All the animals are controlled with hematological and biochemical test, which do not show significant alterations in the standard parameters. The wounds close with little inflammation in the case of ECN and OCN. They have no edges separated, and the tissues throughout the joint areas and nearby are normal. For the wound closed with BCN, the edges are open widely separated from the surface to the bottom, and intense inflammation is present. The skin tissues have structural and inflammatory alterations as a consequence of the BCN stiffness. ECN and OCN present advantages compared with suture: less time for application, immediately hemostasis, no infection, less inflammation and good confrontation of both sides of the incision.

P291 Quantitative Analysis on HeLa Engrafting Ability in NOG Mice

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We conducted quantitative comparisons on xenoengrafting ability between NOG (NOD/*Shi-scid* IL2Rg$^{null)}$ mice and nude mice using HeLa cells which have been used as a positive control material in the WHO TRS878 tumorigenicity test for animal call substraites for the production of biologics. One hundred male NOG mice and 40 male nude mice were assigned to 5, 5 and 4 groups, NOG-HeLa group (The number of the inoculated cells: 0, 10^2, 10^3, 10^4, 10^5 /mouce), NOG-HeLa + Matrigel (MG) group (0, 10^1, 10^2, 10^3, 10^4/mouse) and nude-HeLa group (0, 10^4, 10^5, 10^6/mouse), respectively. Each mouse was inoculated with each prescribed cells subcutaneously in right abdomen and observed nodule formation once a week for sixteen weeks. Tumor formation rates were following: NOG-HeLa group (0: 0%, 10^2 : 0%, 10^3 : 0%, 10^4 : 40%, 10^5 : 100%), NOG-HeLa + MG group (0: 0%, 10^1 : 0%, 10^2 : 60%, 10^3 : 100%, 10^4 : 100%) and nude-HeLa group (0: 0%, 10^4 : 0%, 10^5 : 20%, 10^6 : 70%). The results show that TPD50 (tumor-producing dose at the 50% endopoint) of the HeLa cells mixed with MG in NOG mice was 1/33 ($1.3 \times 10^4/4.2 \times 10^5$) or 1/5431 ($7.8 \times 10^1/4.2 \times 10^5$) of that in the nude mice,

respectively. This suggests that the combination of NOG mice and MG can detect tumor cells with extremely high sensitivity. We are going to show the same data on NOG hairless mice.

P292 The importance of Animal Research in the History of Langerhans Islet Transplantation: Milestone of Langerhans Islet Laboratory

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The role of animal experimentation has been vital in the research and later practical application in therapeutic use. The history of Langerhans Islets transplantation began in 1869, with Paul Langerhans discovering islets in animal models but their function remained unknown. E. L. Opie, in 1901, also using animal models explained the islets function and in 1902 L. W. Ssobolew demonstrated that islets were responsible for sugar distribution. Later in 1904, Jean De Meyer named the products of islets as "insulin". The breakthrough in islet research came in 1965 with S. Moslkalewki employing rats for the isolation and culture of islets. The next significant step was in 1967 by P. E. Lacy and M. Kostianowsky who isolated intact islets from the rat pancreas. In 1971, C. Ricordi and his team started research about the method of islet transplantation from various animals. The next year, P. E. Lacy surgically transplanted islets between animals. In 1988, Ricordi developed the pioneering method for mechanical isolation of large amounts of islets very easily firstly from pigs and later from humans. The most recent development in 2000 in Edmonton, Canada, was when a research team reported seven Type 1 diabetic patients released from daily insulin injections after islet transplantation. Initial phases of this research performed on animals allow for translation of ideas to clinical practice.08.02.2005 Institute Clinical Experimental Medicine, Prague, Czech Republic.

P293 An Alternative Anesthesia Induction Technique in Swine

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An alternative anesthesia induction combination was investigated in female Landrace/Large White swine (n = 46 pigs, 20 ± 3 kg, 10-15 weeks old). The ease of intubation was evaluated by assessing jaw relaxation, resistance to laryngoscope, vocal cord position, vocal cord movement and response to intubation. Pulse oxymetry, heart rate and blood pressure were measured 20 min after premedication with ketamine, midazolam and atropine as well as 5 min after anesthesia induction with a fixed dose of 0,2mg/kg midazolam combined with a dose of 1, 2, 3, 4 or 5μg/kg remifentanil. The majority (93,5%) of the animals were intubated without requiring additional midazolam. One animal developed apnea during intubation and four animals developed electrocardiographic abnormalities. All were resolved without pharmaceutical interventions. Animals that received the dose of 4 and 5μg/kg remifentanil received the best scores in the parameters selected to evaluate the ease of intubation and both had significantly lower post intubation heart rates (P = 0,008, P = 0,032). Animals that received the dose of 4μg/kg had significantly lower systolic blood pressure (P = 0,019). There was no significant difference between the two groups. In conclusion, a combination of 0,2mg/kg midazolam with 4 or 5μg/kg remifentanil may provide an alternative to anesthesia induction for swine. General anesthetics should be readily available and the animal shall preferably be monitored during the procedure.

P294 Antimicrobial Susceptibility of Multidrug-Resistant *Campylobacter coli* and *C. jejuni* from Cynomolgus Monkeys (*Macaca fascicularis*) and Eradication Regimens

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A test for the detection of *Campylobacter* spp. was performed when the cynomolgus monkeys were brought into our center. Since the multidrug-resistant *Campylobacter* spp. were isolated from the cynomolgus monkeys in China, Cambodia and Indonesia (P: which were transferred and bred in Japan), we will report on the antimicrobial susceptibility of these strains and their eradication. Forty-seven among 237 monkeys were infected with *C. coli* and *C. jejuni*. Some monkeys were infected with both species or different strains of the same species. Two monkeys, which were infected with *Campylobacter* spp., showed clinical signs of diarrhea and blood feces. Forty-two strains of *C. coli* and 17 strains of *C. jejuni* were detected. As a result of antimicrobial susceptibility, *C. coli* from the monkey in China was resistant to quinolone, macrolide, tetracycline and gentamicin, and susceptible to chloramphenicol and penicillin. *C. coli* from the monkey in Cambodia was resistant to quinolone, tetracycline and gentamicin, and susceptible to macrolide and penicillin. *C. coli* from the monkey in Indonesia was resistant to quinolone, tetracycline, gentamicin, chloramphenicol and penicillin, and susceptible to macrolide. For the eradication of these isolates, the monkey which was infected with macrolide-susceptible *Campylobacter* spp. was administered azithromycin, the monkey which was infected with gentamicin-susceptible *Campylobacter* spp. was administered gentamicin, and the monkey which was infected with penicillin-susceptible *Campylobacter* spp. was administered amoxicillin/clavulanate. Majority of the monkeys were eradicated. From the perspectives of parasitic zoonosis, we should acknowledge multiresistant *Campylobacter* spp. isolated from the monkeys as a serious warning.

P295 Use of Microcomputed Tomography Characterization in an Osteosarcoma Mouse Model

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Osteosarcoma (OS) is a type of cancer that produces inmature bone. It is the most common type of cancer in bones, and it is usually found at the end of long bones, often around the knee. Frequently it appears in childhood and adolescence. Radiographic and cross-sectional imaging techinques (MR, PET, CT) play a crucial role in the diagnosis of OS. Computed Tomography (CT) has the highest sensitivity and specificity to diagnose and characterize the different kind of OS. The aim of the current study was to evaluate the micro-CT as a noninvasive diagnostic imaging tool in order to study a mice model with OS tumors. For this experiment, 23 mice with tumor induced with the inoculation of OS tumor cells inside tibia bone marrow were used. Each mouse was studied with a high resolution micro-CT eXplore Locus (GEHC) specially designed for laboratory small animal. According to the structure imaged by CT, shape size and density of the affected bone, the mice were classified in three groups: Group A: negative control, normal morphology. Group B: destructive infiltration of bone. Mixed sclerotic and lytic lesion ("sunlight images"). Group C: hypertrophic bone. Increased ossification. Rim of calcification surrounding tumor. Reduction in size of soft tissue mass. The results obtained showed that micro-CT analysis provides valuable noninvasive method for assessment of bone changes in mice models affected by OS. Proposed visual method of analysis is very fast and simple to value morphologic parameters of bone and bone destruction, and could be potentially used in preclinical studies in mice models with osteosarcoma.

P296 Polygamous Intermitted Compared with Permanent Breeding of C57Bl/6J Inbred Mice and Cage Cleaning in Terms of Stress and

Stress-Like Behavior

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Polygamous intermitted breeding of inbred mice is still common use in many institutions, just like the obligatory weekly cage cleaning. Although the general attitude towards these practices is slowly changing, it has neither been proven that polygamous permanent breeding is less stressful nor has it ever been examined how mice really respond to the weekly cage cleaning practice. The purpose of this study was to evaluate these methods. Two groups of mice were compared; one bred permanently, the other intermitted. To evaluate stress levels of C57Bl/6J inbred mice, three methods were used. Firstly, corticosterone metabolites in feces were measured with a 5α-pregnane-3β, 11β, 21-triol-20-one enzyme immunoassay (EIA). To avoid additional stress while collecting feces and still retain individual samples, special cages were developed. The distinctive features of these cages are special feces collecting boxes, fixed permanently to the main cages and separated by sliding doors. The boxes are voluntarily explored by the mice, motivated by food. Secondly, during critical phases of the study, one cage out of each group was monitored by video and the data analyzed by a computer program. Thirdly, the mice were examined on their health status, weight, and behavior at cage cleaning. Even though the third method received inconclusive results, the data collected strongly suggests that polygamous permanent breeding mice had a lower stress level than the mice in the intermitted group and that they are clearly higher after cage cleaning.

P297 Using a Combined Procedure of Rederivation to Eliminate Parasites, Protozoa, Murine Norovirus, *Helicobacter* spp., *Pneumocystis* spp,. and Several Opportunistic Bacteria from a Running Animal Facility

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We combined several procedures to exclude endoparasites, protozoa, Murine norovirus (MNV), *Helicobacter* spp., *Pneumocystis* spp., and several opportunistic bacteria at once from our animal facility in 2012. First of all we sterilized a room with vaporized hydrogen peroxide and facilitated the room with individual ventilated caging system for rederived animals. The cross-foster rederivation was used as basic method. The newborns were seperated from their mother within 24 hours after birth, and rinsed in 50 to 70 ppm of hypochlorous acid (HClO) about 2 minutes, then dried with towel paper and transferred to foster mother's nest. The newborn litter was quarantined for 6 weeks. Fenbendazole containing diet was provided during quarantine. For eliminating opportunistic bacteria, such as *Pasteurella pneumotropica* or *Pasteurella multocida*, enrofloxacin 165 mg/L was added in the drinking water at third to sixth weeks of quarantine. After quarantine, one of the pups and the foster mother were sacrificed for whole panel examination. : ELISA was performed for checking MNV, fecal flotation for pinworms and intestinal protozoa, intestinal contains and trachea flush for bacterial culture, fecal PCR for *Helicobacter* spp., and lung PCR for *Pneumocystis* spp. Around 6 months, 56 litters were rederived by this method, and 4 cased failed to eliminate *Helicobacter* spp., 1 case *Trichomonas*, and 1 case *Pasteurella multocida*. This project is still ongoing. We suggest that this combined procedure can be used to eliminate multiple pathogens efficiently.

P298 Cryopreservation of Mouse Ovaries using High Voltage

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Mouse ovaries can be used to preserve gene resources. However, methods for cryopreservation of full-size ovaries without slicing have not been established. The organs are so large in volume that cell-permeable cryoprotectants cannot penetrate into the tissue. Therefore, ice forms inside the cells during cooling, destroying the cells. However, foods including meats and vegetables have been cryopreserved by application of high voltage. This method is considered to prevent the formation of ice crystals inside the cells. Therefore, we investigated cryopreservation of mice ovaries by application of high voltage. The ovaries of C57BL/6 background mice were cooled to −30 °C at a rate of −1 °C /min with application of 5000 V, and then placed in liquid nitrogen for cryopreservation. For thawing, the ovaries were placed at room temperature and a voltage of 5000 V was applied. The ovaries after thawing were transplanted into same background female mice of the same strain. Ovarian tissue sections obtained three months after transplantation were examined and the cryopreserved ovaries were confirmed, although the number was small. Therefore, we are continuing studying the effects of voltage on ovary cryopreservation.

P299 First Steps of Rus-LASA

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Rus-LASA, Russian Laboratory Animal Science Association was established and registered in the Ministry of Justice of Russian Federation in 2011. The main purpose of Rus-LASA is the dissemination of modern concepts of humane treatment of laboratory animals, and promotion of the harmonization of Russian requirements for laboratory animal care and use with modern European standards. Now there are five working groups in Rus-LASA: on veterinary, on pathomorphology of laboratory animals, on genotypes of laboratory animals, on SOPs and on the translation and publication of subject literature. There are 7 collective and 52 individual memberships from 8 regions of Russia and former Soviet Union. There first annual scientific conference of Rus-LASA was held in Moscow in 2011, the second (2012) - in St. Petersburg, next annual conference will be held in Novosibirsk (September, 2013). The website of Rus-LASA (www. ruslasa. ru), already two years online, is an interactive environment created for educational purposes and for discussion of hot issues Russian LAS practitioners. The website is aimed on the gathering of people professionally involved in biomedical research; it has a public section with general information on laboratory animal care and use and secured part with more extended information. We hope to succeed in improving the quality of science, humane treatment of laboratory animals and creating any clear regulations in Russia. Rus-LASA is a member of Laboratory Animals Ltd. and ICLAS and we are open to further interactions with our colleagues all over the world.

P300 Using Real Time Ultrasonography for the Early Detection and Evaluation of Rat Pregnancy

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Early recognition of rat pregnancy is very important either for breeding or for experimental purposes. The presence of sperm in vaginal smear on the first day (D0) post coitum (p. c.), the sequential measurement of body weight and the palpation of the abdomen, the anatomic changes are some of the methods which are usually used for the recognition of pregnancy. However, the obtained information from those methods is either indicative (sperm in vaginal smear) or delayed

(after the D14 p. c.) and in all cases these are confirmed by birth. The aim of the study was to present our experience from the use of real time ultrasonography during the rat gestation period and demonstrate the quantity and the quality of information which could be obtained not only on the early recognition of pregnancy but also on the assessment of fetal development in the different embryonic stages. A total number of 64 female Wistar rats (HsdOla:WI) aged 16 to 20 weeks were studied. Parameters related to the growth and the overall development of the fetus were evaluated. The presence of embryonic sac was firstly detected on D8 of pregnancy, while more information on anatomy and function of different vital organs were obtained after the D10 of pregnancy. In conclusion real time ultrasonography can detect gestation on D8 and can provide important information on the development of fetuses. This kind of examination is also considered as an alternative method refining the experimental technique and reducing the total number of animals used.

P301 Effect of Repeated Administration of Cyfluthrin on Plasma Monoamines and Malondialdehyde Levels in Rats

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The aim of this study was to determine the plasma monoamines [noradrenaline(NA), adrenaline(A), serotonin(SE)] and malondialdehyde(MDA) levels in rats that were exposed to synthetic pyrethroid pesticide cyfluthrin (14 mg/kg 0.1 × LD_{50}). Sixteen male wistar rats were randomly and equally allocated to experiment and control groups. In the experiment group, rats were treated with cyfluthrin intraperitoneally for 14 consecutive days. Monoamines and MDA levels were analyzed with a high performance liquid chromatography with fluorecence detector (HPLC-FLD). Plasma MDA levels were found as 3.44 ± 0.82, 4.08 ± 1.26 µM on days 0 and 14 in the experiment group, respectively. Plasma MDA levels were found as 2.61 ± 1.43, 3.35 ± 0.67 µM on days 0 and 14 in the control group, respectively. Plasma levels of NA, A, SE were found as 274.74 ± 77.22, 161.24 ± 58.66, 203.51 ± 137.70 µg/L on day 0 in the experiment group, respectively. Plasma levels of NA, A, SE were found as 286.85 ± 113.91, 358.34 ± 313, 155.33 ± 130.17 µg/L on day 0 in the control group, respectively. Plasma levels of NA, A, SE were found as 96.29 ± 83.66, 249.71 ± 374.34 195.70 ± 255.57 µg/L on day 14 in the experiment group, respectively. Plasma levels of NA, A, SE were found as 263.26 ± 164.30, 290.09 ± 66.46, 51.60 ± 31.10 µg/L on day 14 in the control group, respectively. Although an increase of MDA level was observed in cyfluthrin treated rats as compared to controls, the difference was not statistically significant. Furthermore, statistically significant increase was found in plasma SE and decrease in plasma NA and A levels in the experiment group compared to the control group ($P < 0.05$).

P302 Hepatic and Cardiac Effects of Long-Term Sedation with Propofol and Its Vehicle in Rabbits

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In humans, prolonged sedations with propofol or using high doses have been associated with Propofol Infusion Syndrome. The main objective of this study was to evaluate the effects of prolonged administration of propofol in the liver and heart of rabbits. Eighteen New Zealand White rabbits were randomly allocated in three groups that were continuously treated for 20 hours, each group of six animals received: NaCl 0.9% (saline), Smoflipid (vehicle) and Lipuro 2% (propofol). A propofol bolus was given to the propofol group to allow blind orotracheal intubation and mechanical ventilation (100% O_2). Sedation was maintained using infusion rates of: 20, 30, 40, 50, 60 and 70 mg/kg/h. Clinical signs and blood samples were collected every 3 hours. The animals were euthanized, and the liver and heart from each animal were collected for histopathological analysis. Liver changes in animals from the saline and propofol groups were similar and characterized by occasional hepatic cellular tumefaction or mild microvacuolar/hydropic degeneration. In the vehicle group, hepatic lesions of massive hydropic degeneration, consistent with generalized steatosis were observed. The majority of animals tested had no microscopic cardiac lesions, however myocardial vacuolar degeneration, focal necrosis and myocarditis were observed in the propofol group. Our preliminary results suggest that the propofol vehicle induces severe hepatic changes that can be attenuated by the protective effect of propofol in liver tissue. However, propofol administration may pose a higher risk of developing cardiac injury in some individuals, reinforcing the need for assessment of the cardiac function prior to propofol sedation.

P303 Introduction to the International Nomenclature of Laboratory Rodents

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Due to the enormously increasing number of genetically modified laboratory rodents, a clear identification of mutants is mandatory and today more important than ever. Multiple generations of comparable mutations or double imports of mutants are not acceptable for many reasons and should be avoided. However, without following strict rules for the nomenclature it is difficult to correctly identify specific mutant lines. Well-directed funding as well as consortial projects lead to a major increase of double or multiple mutated lines, making correct nomenclature very complex. The international nomenclature rules allow the identification of specific alleles as well as the laboratories, keeping a given line. Only the correct use of the given nomenclature provides a unique identifier for a specific strain, gene, or allele, and thus allows sufficient documentation of experiments and a clear communication with others. In addition, in many countries authorities ask for the use of strain and allele names according to international nomenclature rules. Subsequently, all staff dealing with genetically modified and mutants should be trained to understand and apply these rules. Here, we introduce the basic rules of nomenclature for genetically modified and mutant rodents. We will also give examples from our long lasting experience and we will outline possible complications in cases where nomenclature rules were not used correctly. Possibilities and limitations of the nomenclature rules as well as frequent mistakes will be discussed.

P304 A Tool to Assess Judgement Bias in Mice

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Emotional states are known to affect cognitive processes. For example highly anxious individuals interpret ambiguous stimuli more negatively than low anxious people, an effect called negative judgement bias. Recently, indications were found that judgement bias might be of use to identify emotional states in animals as well. In the present experiments a potential test for judgement bias in mice was examined. We developed a behavioral test set-up that might enable the measurement of judgement bias in mice. Male individuals of the BALB/cJ and 129P3/J inbred mouse strains (two strains potentially differing in anxiety phenotypes) were trained with one odour as a conditioned stimulus (CS+) predicting a palatable almond piece and another odour predicting an unpalatable bitter almond piece (CS-). During testing their reaction times to mixtures of these odours (ambiguous cues) were investigated. Under bright (anxiogenic) as well as dim (anxiolytic) conditions BALB/cJ mice showed an increased latency to pick up the almond piece when exposed to the

CS- (in comparison with the CS+) and showed a comparable reaction to the ambiguous cue, which indicates a negative judgement bias.129P3/J mice did not differentiate between the odour cues. c-Fos expression levels differed between strains and testing conditions in emotion related brain areas. These experiments indicate that this test for judgement bias might be useful to indicate basal emotional trait in BALB/cJ mice. Decreasing negative judgement bias with anxiolytics and the effect of positive manipulation on judgement bias will be subject of further study.

P305 Isolation from Females Modulates Immunity in Male Mice

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The laboratory animal cage housing systems (IVC, ventilated cabinet or isolators) provide excellent protection against pathogens. However the excellent isolation of laboratory rodents from the pathogen limits access to sexually chemosignals. We propose that isolation from chemosignals influence on the results of immunologic studies. The influence of isolation male from female chemosignals on the immunity has been studied in animal facilities (conventional and SPF-grade mouse). Immunomodulation by chemosignals was assessed in the mature males that were kept with or without female scent. Isolation from female chemosignals declined migration of immune cells to skin and airways, synthesis of nonspecific IgGs and sIgA and platelets. Reduction of WBC in airway in males kept without female signals results in increasing susceptibility to murine flu virus A/WSN/33. Other series of our study have been concerned demonstration of influence on specific immunity in males of isolation from female scent. In contrast, chemosignals prohibition increased B/T ratio in peripheral blood, production of antibodies and proliferation activity of splenocytes in response to mitogen (LPS). We have shown that changes in specific immunity associated with female chemosignals can influence on effectiveness of vaccination and development of an allergy. Thus the isolation male mice from female chemosignals redistributed the immunity in favor of specific. Immunmodulation by chemosignals must be considered to plan of immunologic experiments. Ordinary replacement of housing units with open cages to IVC could significantly change the results of immunologic tests.

P306 Analytic Performance of Both Hematology Analyzer and Flow Cytometer Systems for the Phenotyping of Peripheral Blood Leukocytes in Inbreed and Outbreed Mouse Strains

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Flow Cytometry (FC) provides significant information on cellular and sub-cellular levels for Immunology and Hematology. FC allows precise multiparameter enumeration of leukocytes populations because it is a sensitive, rapid and less operator-dependent technology. Currently, Hematology analyzers (HA) are being used in research as a complement technology to FC because of the amount of data obtained from whole blood samples. Several studies have been conducted to establish the accuracy and reliability between both systems. Data about their usefulness is scarce in laboratory animals. This study analyzed the reliability of 5-part differential leukocyte counts by both state-of-arts HA and FC in 3 inbreed mouse strains and an outbreed mouse stock. When each leukocyte population was analyzed, a statistically significant difference between both FC and HA quantifications was found. Leukocyte estimation disparity could be due to the cell membrane complexity and the heterogeneity of it found between strains. FC gives greater advantage for leukocyte discrimination than HA because of analysis rigidity of this last. We found that the accuracy of both analyzers is inversely proportional to the leukocyte membrane complexity exhibited for each mouse strain. In conclusion, FC and HA are useful and complementary tools for phenotyping studies in laboratory animal science, keeping in mind the phenotypic features of each mouse strain.

P307 Comparasion of the Sedative Effects of Xylazine, Romifidine, Medetomidine and Dexmedetomidine in Mice during Embryo Transfer Procedure

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The objective of this study was to compare the sedative effects, intraoperative inhalant anesthesia required and recovery times of four different α(2)-adrenergic agonists in mice during routine surgical embryo transfer procedures. This pilot blinded prospective study included 15 adult female CD-1 ICR mice ($n = 3$/group). Mice were premedicated intraperitoneally (IP) with injection of either xylazine (XYL; 5 mg/kg), romifidine (ROM; 0.75 mg/kg), medetomidine (MED; 0.5 mg/kg), dexmedetomidine (DEX; 0.25 mg/kg) or saline (control group). Premedication also included a bolus of meloxicam (2 mg/kg) and buprenorphine (0.1 mg/kg) IP. Anesthesia maintenance was performed with isoflurane by mask. Atipamezol (5 mg/kg) IP was administered before surgery ended. Behavioral changes were observed and video-recorded. Spontaneous locomotor activity (ALE) was evaluated after premedication and during anesthesia recovery for evaluation of quality of sedation. Time from premedication to induction and quality of sedation, withdrawal loss test, isoflurane vol% during maintenance and time to recovery were recorded and compared between groups using nonparametric tests. $P < 0.05$ was considered significant. All α(2)-adrenergic agonists caused significant sedative effects compared to the control group ($P = 0.002$). Quality of sedation, time to induction, withdrawal loss test and time to recovery were not different between α(2)-adrenergic agonists. Isoflurane vol% was significantly lower for medetomidine and dexmedetomidine compared to the other tested groups ($P = 0.003$). No differences were found in time to induction, quality of sedation and time to recovery between α(2)-adrenergic agonists, however medetomidine and dexmedetomidine decreased maintenance anaesthetic inhalant requirement during surgical embryo transfer procedures in mice.

P308 A Comparative Study of the Sensitivity of the Bühler Test and the Maximization Test in Experimental Studies on Dermal Allergenicity in Guinea Pigs

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One of the common health problems and a major cause of occupational dermatoses is allergic contact dermatitis. There is a need to identify skin sensitizing chemicals resulting from two major aims of predictive testing for contact allergens - the safety of workers in chemical production sites and users of end product coming in contact with the skin. Various methods to identify the allergic potential of chemicals are available; however, the OECD recommended the Maximization Test of Magnusson and Kligman (MT) in which sensitization is potentiated by the injection of Freund's Complete Adjuvant (FCA) and the Bühler Test

(BT) without the adjuvant injection as primary methods. The study was performed according to the OECD Guideline for Testing of Chemicals No.406 and the EU Method B.6: Skin sensitization. Sensitization was induced by using a substance which is known to be mild-to-moderate sensitizer. After termination of the experiment and the macroscopic evaluation of the treated skin, the microscopic examination of spleen, submandibular lymph node and treated and untreated skin was conducted. Tissues were fixed in 10% formalin, and stained with haematoxylin and eosin. The study showed that the Maximization Test is more sensitive than the Bühler Test though the histopathological examination does not explain changes in organs due to the proinflammatory activity of FCA. It seems that the BT better reflects the real exposure to substances than the MT. Using the BT, one may avoid overestimating potentially very weak allergic substances; however, there is also the possibility to underestimate potentially strong allergens. It seems that the BT better reflects the real exposure to substances than the MT. Using the BT, one may avoid overestimating potentially very weak allergic substances; however, there is also the possibility to underestimate potentially strong allergens.

P309 Limitations in the Expression of EGFP in Retinal Cells of Green Mice

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Many cell transplantation studies have been performed using transgenic mice with an "enhanced" green fluorescent protein (EGFP). Although such "green mice" (GM) were considered as a source of ubiquitous green cells, the level of EGFP expression varies among organs and tissues. We show here the differential expression of EGFP into the retina of GM. Heterozygous GM [C57BL/6-Tg(CAG-EGFP), $n = 2$] were used and compared to three control mice: a not-green mouse obtained from the same crossing, a wildtype C57BL/6J mouse, and a Swiss mouse. Animals were 3-4 months old and all except one green mouse were anesthetized using ketamine plus xylacine and then exsanguinated and subjected to intravenous perfusion with paraformaldehyde. The remaining green mouse was not perfunded, but euthanatized by injection of anesthetic overdose. Retinal criosections and whole mount retinas were analyzed by direct observation by confocal laser scanning microscopy and immunocytochemistry, using specific retinal cell markers. In the two GM, we found that many EGFP-expressing cells were located in the outer retina: in retinal pigment epithelial cells, in the photoreceptor layer, in the outer plexiform layer, and in some bipolar cells in the inner nuclear layer. However, cells in the inner retinal (about 50% depth in the inner nuclear layer, in the inner plexiform layer and in the ganglion cell layer) were not green: amacrine, some bipolar cells and ganglion cells did not express EGFP in GM retina. Differential retinal expression of EGFP in "green mice" demonstrated limitations of GFP as a cell lineage marker or donor origin.

P310 Are all the Glucose Meters the Same? ISO-Based Assessment of Accuracy and Precision in Dogs

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Portable blood glucose meters (PBGMs) for humans are often used in dogs both for research and clinical purposes, without considering potential inaccuracy. Accuracy and precision of 9 PBGMs were assessed in canine plasma, based on the ISO 15197:2003, and results were compared with those previously published in whole blood. We evaluated: GlucocardG Meter; Onetouch Vita, VerioPro and UltraEasy; Optium Xceed, Hemocue Glucose201, Accuchek AvivaNano, Freestyle FreedomLite, StatStrip Xpress. Samples were collected from our Veterinary Hospital´s patients. A multilayered, dry-slide technology, hexokinase method was used as reference. To assess accuracy and precision, PBGMs and reference concentrations were compared (Student's t for paired data and Bland-Altman plots) and intrassay variation coefficients (CV) were calculated, respectively. One hundred samples were analyzed for accuracy and 23 for precision (29-579 mg/dl). Unlike what was observed for whole blood, in plasma, most PBGMs showed significantly higher values than the reference (175.30 mg/dl, SD 115.74). On average, results from FreestyleLite (174.41 mg/dl, SD 111.70) and StatStrip (179.22 mg/dl, SD 129.16) were similar to the reference ($P > 0.14$). AvivaNano, OneTouch VerioPro and FreestyleLite achieved (^{3}95% of values within the recommended limits) and StatStrip approached (92%) the ISO accuracy requirements in plasma. Precision improved for most PBGMs when plasma was used (mean CV 4.05%, SD 1.21) instead of whole blood (mean CV 7.44%, SD 2.07). Thus, according to our previous and present results, different PBGMs should be considered in canine glucose studies, depending on the type of sample to be used and whether precision or accuracy are the priority.

P311 A Single Intraperitoneal Injection of Different Doses of Ketamine Did Not Induced Acute Hepatic Injury In Adult Mice

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Ketamine is an effective anaesthetic and analgesic agent used frequently in laboratory animals. It was described that it has hepatic metabolic implications, however little is known about the secondary effects of a single administration of this drug on liver. Therefore, we studied the effects of different doses of ketamine on liver in adult mice. Thirty-two C57BL/6 adult mice were divided into 4 different groups (Saline solution, ketamine 25 mg kg^{-1}, ketamine 75 mg kg^{-1} and ketamine 150 mg kg^{-1}). Eight animals per group were sacrificed, 3 hours after anesthesia, and used for histopathological (haematoxylin-eosin staining) and immunohistochemical analyses (activated caspase-3 detection). Hepatic injury was graded by histologic examination and by counting the number of hepatocytes with positive reactivity to activated caspase-3 antibody. No significant differences were detected between groups regarding the hepatic histopathologic lesions such as necrosis or vacuolization of hepatocytes, and regarding the number of apoptotic cells. These findings showed that a single intraperitoneal administration of different doses of ketamine did not induce necrosis or apoptotic degeneration on liver of adult mice 3 hours after after anesthesia, suggesting that ketamine can be used in adult mice without induce acute lesions on liver.

P312 A Single Intraperitoneal Injection of Ketamine Did Not Affect Hippocampal Long-Term Potentiation of Adult Mice

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imbra, Coimbra, Portugal; [6]CenterEffects of ketamine/medetomidine combination on basal synaptic transmission and synaptic plasticity in hippocampal slices of adult mice for Neuroscience in Cell Biology, University of Coimbra, Coimbra, Portugal
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Ketamine is frequently used to induce analgesia or anesthesia during research procedures with animals. However, little is known about its effect on memory and learning. Long-term potentiation (LTP) is considered a cellular mechanism for learning and memory. Therefore we investigated the effects of different concentrations of ketamine on long-term potentiation. Eight C57BL/6 male adult mice were divided into 3 different groups (Saline solution, 25mg kg-1 ketamine, 75mg kg-1 ketamine). Twenty-four hours after injection, animals were euthanized and hippocampal slices were used to record the evoked field excitatory postsynaptic potentials (fEPSP). High-frequency stimulation (100 pulses at 100 Hz) was used for LTP induction. The initial slope of the fEPSP was measured and LTP induction and maintenance were calculated. The data were analyzed using one-way ANOVA followed by Bonferroni post hoc tests. No significant differences were detected between groups regarding the induction or maintenance of the long-term potentiation. This finding suggests that a single intraperitoneal injection of ketamine did not affect hippocampal long-term potentiation 24 after injection, suggesting that ketamine did not induced deficits of memory in male adult mice.

P313 Effects of Ketamine/Medetomidine Combination on Basal Synaptic Transmission and Synaptic Plasticity in Hippocampal Slices of Adult Mice

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The ketamine/medetomidine anaesthetic combination is frequently used in research procedures with animals and routine veterinary medicine. However, there is a lack of information about its effect on hippocampal synaptic activity. This activity is essential for learning and memory formation. Therefore we investigated the effects of ketamine/medetomidine combination on basal synaptic transmission and on two forms of synaptic plasticity (paired-pulse facilitation (PPF) and long-term potentiation (LTP)) in the CA1 region of mouse hippocampal slices. Evoked field excitatory postsynaptic potentials (fEPSP) were recorded CA1 pyramidal cell synapses of mouse hippocampal slices. Three slices per group and experiment were used. For basal synaptic transmission and PPF, increasing concentrations of ketamine combined with medetomidine: 30+1.2, 100+4 and 200+8 (µM of ketamine + medetomidine, respectively) were applied in hippocampal slices. For LTP experiments, 3 µM of ketamine combined with 0.12 µM of medetomidine were applied to slices. LTP was induced by high-frequency stimulation. The synaptic transmission strength was assessed by measuring the initial slope of the fEPSP. LTP induction and maintenance were calculated. PPF was estimated as the ratio between the slopes of the second and first paired pulses. Ketamine/medetomidine combination did not affected PPF neither basal excitatory synaptic transmission but decreased LTP in the CA1 region of the mouse hippocampus. These findings suggest that ketamine/medetomidine combination may induce deficits in memory after anesthesia mainly by alteration of postsynaptic mechanisms involved in LTP.

P314 Follow Up of Neonatal Mice Injected with Streptozotocin

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Repeated low doses administration of streptozotocin (STZ) is widely used to induce insulin dependent diabetes mellitus in mice. We have recently adapted this method to use it with neonatal mice. Now, our aim was to determine the long-term effect of STZ injection in neonatal mice. Hsd:ICR(CD-1) mice were used. STZ was dissolved in 0.1 M citrate buffer (pH 4.5) to a 5 mg/m concentration just before use. Newborn mice received three STZ (40 mg/Kg) intraperitoneal injections at day P3, P4 and P8. A welfare supervision protocol approved by the CEEA of the UAB was implemented. Thus, weight, color, activity and presence of milk spot were determined in pups daily. Appearance, relative size, coat condition, posture, activity and clinical signs were checked daily later. Body weight and blood glucose were determined weekly after weaning. Humane end points were established. Mice were euthanasied at 4, 12 and 24 weeks and a histopathological study of liver, pancreas and kidney was done. STZ treated mice presented hyperglycaemia (363 ± 63 mg/dl) by day P26. However a clear sex effect was detected. Blood glucose levels increased in males up to 588 ± 28 mg/dl by P54 and remained high for the rest of the study. However, hyperglycaemia in females arrived to a maximum of 476 ± 119 mg/dl by P54 and showed a tendency towards normoglycemia by week 24. Neoplastic lesions were found in the livers of all STZ treated mice.

P315 Effects of Thermal Sterilization on Selected Components of Diets for Laboratory Rodents

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The objective of the study was to assess changes in contents of nutrients and antinutrients in sterilized rat diets as measures corroborating in vivo studies on the choice of a less destructive autoclaving program. Two cereal –based diets containing either soybean meal (S) or casein (C) as protein supplements and a commercial autoclavable diet (SN) of unknown composition, were sterilized by autoclaving at 121°C during 20 minutes (T1) or at 134°C during 10 minutes (T2), control diets were not autoclaved (T0). Autoclaving did not affect macronutrient contents including minerals and nonphytate to total phosphate proportions. In all diets it increased the neutral detergent fiber (NDF) content and amount of protein bound to NDF. Losses of particular vitamins differed among the diets and treatments: among water soluble vitamins the most stable was thiamine, riboflavine and pyridoxine whereas among fat soluble – vitamin E, and the least stable A and D. The increase of acrylamide concentration due to autoclaving was the greatest in S and the smallest in SN diet. Autoclaving reduced the total isoflavones (phytoestrogen) concentration in S diet to a small degree but was more effective in destruction of two particular minor components. While the effects of autoclaving differed to some extent among the diets, they were on general less pronounced when diets were autoclaved at lower temperature during longer time (T1) than shortly at higher temperature (T2). It was therefore concluded that the T1 program should be recommended as less detrimental. Financial support project no. NR12003506.

P316 The 3Rs-Center Utrecht Life Sciences

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According to the Directive 2010/63/EU, each EU Member State shall establish a national committee for the protection of animals used for

scientific purposes. The Utrecht University (UU) and University Medical Center Utrecht (UMCU) have established a local center for the protection of research animals: *the 3Rs-Center Utrecht Life Sciences*. The aim of the Center is to stimulate the development, acceptation and implementation of replacement, reduction and refinement methods (3Rs) within the Utrecht Life Sciences (ULS), to communicate about this and to build and maintain a network of 3Rs experts within and beyond the ULS.

The 3Rs-Center ULS activities involve: (internal and external communication regarding animal experiments and 3Rs; contribution to the publication of the UU/UMCU annual report on animal experiments); (providing lectures on 3Rs in the Laboratory Animal Sciences course and on invitation; informing high school biology teachers on the 3Rs); (regular internal newsletter; website: *www.uu.nl/3RsCenterULS*; twitter: *@3VCentrumULS*), and (animal ethics committees; ULS institutes, for example, on the consequences of the Directive 2010/63/EU on research and education; researchers on issues related to animal experiments and related to 3Rs; Animal Welfare Body on 3Rs matters). Members of the Center participate in the activities of the Netherlands Knowledge Center on Alternatives (NKCA) and have positions in national and international 3Rs organisations. The Center cooperates with research organisations at the Utrecht Science Park and with other Utrecht Life Science partners.

P317 Formulation and Evaluation of an Autoclavable Low-Phytoestrogen Diet for Rats

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Soybean meal is commonly used as a protein supplement in rodent chows but is also an important source of phytoestrogens which affect animal physiology and distort outcome of the experiments. The commercial low-phytoestrogen soya-free diets are generally not provided with the information on their protein content and on the recommended sterilization treatment. The objective of the study was to formulate a low-phytoestrogen diet supplemented with casein and to adopt sterilization program based on its effects on the composition and nutritional value of the diet. Two cereal-based diets supplemented either with soybean meal (S) or casein (C) (or sodium caseinate) were sterilized by autoclaving at 121°C during 20 min (T1) or at 134°C during 10 min (T2), control diets were not autoclaved. Sterilization induced small decrease of dietary vitamin contents and considerable increase of acrylamide concentration, smaller in C than in S diet. In both diets it caused some deterioration of protein digestibility and biologic value. The negative effects of autoclaving were less pronounced at T1 than T2 program. On general, the indices of protein and energy value, growth performance and some analyzed physiologic and biochemical parameters indicate that low-phytoestrogen diet supplemented with casein or sodium caseinate has nutritional value equivalent to that of soybean containing diet and is fully autoclavable, preferably according to T1 program. Financial support project no. NR12003506.

P318 Creating New Insights from Data Gathered during Animal Research through Data Sharing and Cross-Analysis

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In 2011, an extensive review of internal practices in relation to animal research was conducted at a global organisation. This together with external benchmarking led to a new four-fold animal research strategy endorsed by the Company Executive Team. The four pillars of this strategy consist of building on our committment to the development of innovative alternatives to animal research, optimising animal studies through enhanced scientific review, finding ways to maximise and harness the use of our research data, and bolstering our external collaborations in this area. As part of the implementation of this strategy, a multidisciplinary team of biologists, statisticians, and information techonology experts explored new ways of capturing, accessing and cross-analysing historical data generated in different parts of the organisation. The aim was to facilitate the optimisation of animal models of efficacy, to better understand their value in the drug discovery process, and eventually to pave the way for easy and cost-effective external sharing of data generated from animal research. In this poster the challenges associated with changing the information management process, including the cultural aspects, within a large research organisation will be discussed and customer-focused solutions addresssing these challenges will be presented. The investigation showed the benefit of capturing animal model information in a structured yet flexible environment, and demonstrated how the ability of modern statistic programs to speak directly to structured data sources can be leveraged to make available cross-study statistical analysis, thereby enabling better understandings and new scientific insights from animal research data.

P319 Blood Collection and Reference Range of Hematology and Blood Chemistry Parameters in Chinchilla Lanigera

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Although chinchillas are commonly used in research as otology models, we have found limited amount of literature about physiologic parameters and other basic experimental methods. The purpose of this work was to establish a reference range for the most common blood parameters and to learn more about the anatomy, handling and behavior of this species during the experimental process. The samples were obtained from the jugular vein of five chinchillas (males, eight months old, weight range: 400 to 500 g). These animals are quiet and very docile, and it is possible to take blood samples with a slight restrain without anesthesia. We used a 25 gauge needle attached to a 1-ml syringe. A maximum blood volume of 3 ml was withdrawn from each animal.2 ml were drawn into a tube with anticoagulant for hematology and 1 ml in a tube without anticoagulant for blood chemistry analyses. High glucose, GOT, and LDH levels were observed. The high level of glucose was probably caused by nonfasting of the chinchillas. LDH could be due to slight samples of hemolyzation, and, for GOT, we do not have a complete explanation yet. It was also confirmed the high lymphocytic percentage (80% of the total white cell), in this species. In conclusion, we have established a reference range for common blood parameters, with a procedure without stress or pain for the animals. More analysis will be necessary to reach a conclusion about GOT and LDH values.

P320 ICLAS Performance Evaluation Program for Diagnostic Laboratories (PEP): A Robust Scientific Tool to Enable Diagnostic Laboratories to Monitor Their Diagnostic Performance

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The Performance Evaluation Program for Diagnostic Laboratories (PEP) was set up in 2007 to promote and maintain the use of high quality animal models in research by providing a tool for diagnostic laboratories worldwide to monitor the sensitivity and specificity of their health monitoring assays. Serum and microbiological specimens are prepared and confirmed by internationally recognized laboratories and sent to subscribing participating laboratories around the world for analysis.

All samples are generated under stringent conditions and extensively characterized. Agents are obtained from known sources and identified phenotypically and genotypically to confirm identity. The masked specimens are sent to participating laboratories as unknown samples. The current PEP specimen library includes all common agents that can infect rats and mice. Batches of 10 serum and/or microbiological specimens are sent annually to subscribing participating laboratories around the world for analysis. Participating laboratories then request expected results and a comparison of results enables them to self-asses their diagnostic performance. There are currently 20 participating laboratories from: Asia (5), Australia (2), Europe (7), North America (5) and South America (1). For participating laboratories the main benefits of participating in PEP are: (1), use of a scientifically robust program to monitor diagnostic performance; (2), access to expert help and advice from internationally recognized diagnostic laboratories, (3), participation in PEP enables the labs to implement a quality assurance program and (4), the labs contribute to improved animal quality in research institutions. Details of PEP can be found at http://iclas. org/animal-quality-network [1] Financially supported by grant ACI2008-0829 from MICINN. http://iclas. org/animal-quality-network.

P321 EUPRIM-Net II: European Primate Network - Advancing 3Rs and International Standards in Biologic and Biomedical Research

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Now and for the foreseeable future nonhuman primates (NHPs) will play an irreplaceable role in biologic and biomedical research where their use can be sufficiently justified and no alternatives exist. Although only 0.1% of all animals used in basic or applied research are NHP, their high sensory and cognitive abilities necessitate that research with NHP is subject to particularly high ethical (and legal) standards. A careful application of the 3Rs concept of Refinement, Reduction, Replacement must be imperative. In 2006 the European Commission provided funding to EUPRIM-Net. The Research Infrastructure which links nine primate centers in six European countries is now in its second round of funding until 2014. Under this project, the primate centers' infrastructures and expertise are integrated to provide critical services, training and advice to scientific institutions in Europe conducting primate research and to zoological gardens keeping primates. The activities are divided into Network-, Access- and Research Activities all aimed at promoting animal welfare and the 3Rs. Since 2011, the project actively develops ties to the industry and primate centers outside EU borders. Directive 2010/63/EC foresees various animal protection and welfare measures reflected in EUPRIM-Net's activities. The Network Activities are about Education and Training, Best Practice and Veterinary Care, Positive Reinforcement Training (PRT) and Animal Behavioral Management. These activities are supported by Research Activities on Diagnostics and Diseases, Telemetry, and Alternative Methods. Moreover, EUPRIM-Net offers access to primate material (BioBank) and to primate-based animal models of severe human diseases (PRIMOCID) thus contributing to improving the 3Rs.

P322 Age-Related Changes of Physiologic Parameters in Young Adult Rats: A Noninvasive Observational Study (Preliminary Results)

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The aim of this study was to investigate changes of physiologic parameters in laboratory rats during their first year of age fed ad libitum. Forty 16-week-old intact female Wistar rats were recruited for this licensed study. Rats' body weights (bw) were measured twice monthly and food and water consumption was calculated twice weekly per 100 g bw/day/rat, preliminarily reported here from 4 to 6 months of age. Haemodynamic parameters were evaluated by a noninvasive blood pressure (BP) instrument (NIBP Panlab Harvard Apparatus LE 5002) at 4 and 6 months of age, in which the rats, after acclimatization to the procedure, were restrained in a heated cylindrical holder with a tail pressure-cuff applied. Mean haemodynamic values were calculated from 6 consecutive measurements. Body weight increased from 258.95 ± 20.38 (mean ± SD) to 273.40 ± 21.69 g [*P* nonsignificant (NS)]. Food consumption changed from 7.56 ± 1.02 to 7.18 ± 0.75 g per 100 bw/day/rat (p NS). Water consumption changed from 11.67 ± 2.39 to 12.20 ± 1.92 mL per 100 bw/day/rat (p NS). The mean (± SD) heart rate changed from 412.73 ± 40.51 to 373.96 ± 18.75 bpm, systolic blood pressure (bp) 123.38 ± 14.17 to 131.69 ± 14.26 mm Hg, and diastolic bp 99.11 ± 9.47 to 105.75 ± 14.98 mm Hg respectively from 4 to 6 months (all p NS). A decrease of heart rate and increases of bps were noted. These ongoing measurements will allow us to examine potential bw and haemodynamic parameters' correlation, regarding possible metabolic syndrome appearance with aging.

P323 Assessing the Suitability of the Compatible Solute Ectoine to Improve Cryopreservation Protocols in ARTs

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With increasing numbers of genetically modified mouse lines assisted reproductive technologies (ARTs, that is,, cryopreservation, in vitro fertilization (IVF), embryo transfer) have become indispensable in modern animal facilities. Cryopreservation enables archiving of mouse lines. Efforts are made to reduce formation of ice crystals by using cryoprotectants. However, DNA degradation during the freezing and thawing process still limits the rederivation success. In an earlier experiment, the compatible solute ectoine which enables microorganisms to survive extreme conditions improved the cryopreservation and rederivation success of pronucleus-stage embryos. We examined the effects of five different ectoine dosages added to the cryoprotectant on the cryopreservation of embryos and sperm in three different mouse strains. The best dosage for embryo freezing for each strain was determined by the thawing rate. If there was a significant improvement of thawing rate with a given dosage the effect of ectoine on the viability was then examined by embryo transfer and the success of rederivation was determined. The most efficient ectoine dosage for sperm freezing was determined by measuring motility and progression of frozen/thawed sperm and subsequently tested in IVF. Only in C57BL/6J, there was a significant improvement of the thawing rate with ectoine. However, the success of rederivation in this group was significantly reduced. For sperm freezing ectoine improves motility and progression in all three mouse strains, but there was no positive effect in fertilization rates. Therefore the use of ectoine in ARTs is not recommended with the current protocol.

P324 The Benefits of a One Health Approach to Comparative and Translational Medicine in an Australian Research Institution

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The One Health strategy, involving the study of disease processes across species, is operating at different levels within many laboratory animal research institutions and can be successful when the fundamental need for interdisciplinary collaboration between human and animal health care professionals is active. This approach is demonstrated in this presentation where the strengths and shortcomings of One Health are described through in vivo examples and translational medicine within a large Australian university institution. Overseeing the animal care and use across this institution and medical facilities by comparative

medicine veterinarians relies on direct communication, team work and a complex network of associations that often underpins the successful outcomes of valid scientific outcomes. The importance of good animal health monitoring, reducing environmental stresses and striving for best practice in animal care and use will be described. The variability between animal facilities within this institution and the challenges that result are discussed. Case examples are used to illustrate the importance of evidence-based laboratory animal science and the role that comparative medicine veterinarians and animal technicians play in the One Health approach to translational medicine. By assessing models of cancer, epilepsy, spinal cord injury, cardiovascular disease, Alzheimer, Parkinson disease and vaccine development and the institutional interdisciplinary approach, much can be shared and learnt to establish improved animal care and valid scientific outcomes. It is proposed that as this institution embraces globalization and collaborative international projects the importance of a One Health approach will become more evident.

P325 Are "Semi-Chemically-Defined" Diets for Mice a Means to Reduce Variations of Results?

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Standard natural-ingredient diets of commercial suppliers fulfil the nutritional requirements for must mouse strains. However, it is nearly impossible to achieve consistency for any ingredients and the diets vary from batch to batch. Natural-ingredient diets may also contain contaminants (for example, microorganisms, parts of microorganisms, mycotoxines, pesticides, secondary plant products), which can influence the outcome of experiments. Together with Altromin we developed "semi-chemically-defined"diets to solve this problem. The nutrient level of a standard natural-ingredient-diet was used as basis. The "semi-chemically-defined"diets were free of nitrosamines, gluten, aflatoxine, phytoestrogen, soya, and toxic matters. Five diets were tested: a natural-ingredient standard diet (5% fat, 22.5% protein), two "semi-chemically-defined" diets with 25% protein and 10% and 14% fat respectively, and two "semi-chemically-defined" diets with 19% protein and 10% and 14% fat respectively. The "semi-chemically-defined" diets were composed from rapeseed oil, maze-protein, maze-starch, cellulose, and sucrose. The study included mice from the P0-generation, F1-generation, and F2-generation of BALB/cJ, C57BL/6J, C3H/HeJ, NMRI. Weight gain, reproductive performance, histologic analysis, gross morphologic examinations, and clinical-chemical analysis were made from F2 animals to guarantee that the results were exclusively due to the experimental diets. We found differences in all parameters between the tested strains. However, the differences were strain specific and not a result of the diet. In summary we showed, that our "semi-chemically-defined" diets fulfil all nutritional requirements. However, they had not reduced the result-variances for the parameters we examined. But they might be an inexpensive alternative for the expensive "total chemically defined" diets.

P326 In Vivo Effect of Herbal Extracts (*Nigella sativa, Zingiber officinale*) Alone and Combined with Primethamine-Sulfadiazine against *Toxoplasma gondii*

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Toxoplasmosis is a zoonotic infection that caused by *Toxoplasma gondii.* The aim of this study was to investigate the effects of adding two different immunomodulatory agents, *Nigella sativa* or *Zingiber officinale*, to the classic therapeutic agents, pyrimethamine and sulphadiazine, in combination, to support immune system, which is very important in toxoplasmosis, and on the survival of mice. In our study, Balb/c mice were infected ($n = 135$) by 10^5 *T. gondii* tachyzoites intraperitoneally and treatment was begun after the first 24 hours ($n = 126$). The total number of groups were 15 (1 infection control group, 4 treatment control groups, 10 test groups). Each group contained 9 mice. No drugs were used in the infection control group. All drugs were continued for 10 days. After the mice were infected, they were observed for 30 days and their life time was evaluated. When we evaluated our findings it has been seen that the survival rate at the pyrimethamine 6.25 + sulphadiazine 100 mg/kg/day and 400 to 200 mg/kg/day *Zingiber officinale* or 1700-850 mg/kg/day *Nigella sativa* and pyrimethamine 12.5 + sulphadiazine 200 and 400 mg/kg/day *Zingiber officinale* combination group was increased and it has been found statistically significant ($P < 0.05$). In conclusion, our findings considered that, combination of PYR-SDZ with *Zingiber officinale* or *Nigella sativa* as an alternative choice in the treatment. Further in vivo studies involving different agent combinations under specified dosage regimens are needed to demonstrate the effects of which the active ingredients of herbal extracts agents in the treatment of *T. gondii* infections.

P327 A Comparative Phenotypic Analysis of 2 C57BL/6N Substrains

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While the mouse inbred line C57BL/6J is widely used in mouse genetics, the International Knockout Mouse Consortium (IKMC) has established a mouse ES cell mutant resource employing C57BL/6N ES cells. A number of independent C57BL/6N subtrains being available from various animal providers, genetic differences accumulated over the years which likely may contribute to phenotypic variation. Investigators may thus ask if some phenotypic effect variation of the mutation could exist depending on the C57BL/6N substrain background used for establishing mutant mice colonies. To address this question, we subjected C57BL/6NCrl and C57BL/6NTac to a comprehensive phenotypic analysis. We used the phenotyping test battery developed by the International Mouse Phenotyping Consortium, namely the ImPRESS protocol (http://www.mousephenotype.org/impress). In our design study the C57BL/6NTac substrain was bred internally and we compared them to C57BL/6NCrl either provided by the vendor at 6 weeks of age, or bred in house. In summary, we were able to show that there were no significant phenotypic differences between both substrains, as long as both colonies are bred under the same husbandry conditions. In contrast, some phenotypic parameters were significantly different when C57BL/6NCrl mice are provided by the vendor at 6 weeks of age, in comparison to internal breeding. In conclusion, with regard to the IKMC ES cell resources and mutants phenotyping studies, the two C57BL/6N substrains can be used for colony breeding, giving reliable phenotyping results as long as mutant and control mice are set up in the same environmental conditions.

P328 Inhibition of Stat3 in Cancer and Chronic Inflammation

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Members of signal transducers and activators of transcription (STAT) family have been found to be constitutive activated in a wide range of human tumors. Stat3 is present in its activated form in breast- or prostate cancer. In addition to its important role in solid tumors Stat3 is also known to be activated in chronic inflammation (for example, psoriasis). Since Stat proteins do not entail enzymatic activities, they have been

considered as nondruggable. We are developing novel approaches for the derivation of Stat inhibitors and devised peptides able to mask defined functional domains of Stat3. These peptides are able to specifically inhibit their function and affect Stat addicted tumor cells in vivo. We successfully identified a peptide aptamer, which inhibits Stat3 in tumor models (4T1 mammary tumors) in mice. For experimentally influence of chronic inflammation we establish a mouse model for psoriasis using imiquimod (a ligand of TLR7) as an inducer of systemic inflammation. This inflammation resembles several features of psoriasis in mice, including activation of Stat3. We have shown in previous studies that this model serves as a very elegant tool for investigating the effects of Stat3 inhibitors in the context of chronic inflammation in vivo. Our in vivo studies results in reduction of tumor growth using Stat3 inhibitors like the peptide aptamer rS3-PA and low molecular weight compounds targeting Stat3. Also therapeutic effects we observed in the model of chronic inflammation in mice. Inflammatory signs like increased spleen weight could be cured using the inhibitors.

P329 Analysis of the Effects of Inbred Breeding *Phodopus sungorus*

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Inbred strains are one of the most widely used models in experiments on laboratory animals. They are more stable, more uniform, more repeatable, and better defined than outbred stocks. Phodopus sungorus is frequently used for studies addressing the regulation of fertility by environmental factors such as the photoperiod and the temperature. We have attempted to obtain an inbred strain of Phodopus sungorus. The research material are animals kept in the Animal House of the Faculty of Biology, University of Warsaw, from 2003 up to now. They derived from the outbred stock. Now we have 15thgeneration of brother-sister matings. Level of inbreeding is 0.96. We analyzed the effects of inbred breeding.

P330 ForSaTum: An animal Research Platform for the Longitudinal Analysis of Novel Cancer Treatment Concepts Supporting the 3R Concept

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Although the development of cancer therapy has achieved considerable success, less than 10 out of 10,000 cancer drug candidates undergo clinical trials, whereas 80% usually don't pass the final clinical test. One of the main reasons for this is the lack of efficient pre-clinical facilities for standardized longitudinal studies of new cancer drugs in relevant tumor models. Here we show a unique specialized animal research platform (ForSaTum) providing a counsel service for experimental animal work and performing pharmacokinetic and toxicology studies according to OECD and ICH guidelines under GLP standards. Thereby, best suitable tumor models are selected, established and tumor tissues are cryoconserved for further analysis. For longitudinal studies of new cancer diagnostics and therapeutics the program provides several molecular imaging technologies such as computer tomography (CT), ultrasound (US), mPET, optical and magnetic resonance imaging (MRI). In addition, an IT platform containing a comprehensive data base, a work-flow based study editor, and tools for intelligent information extraction ensures the quality and documentation of results and meets the requirements needed for acceptance in clinical testing. The longitudinal analysis will enhance the quality of pre-clinical studies and subsequently the success of clinical trials. Furthermore, this concept has the potential to refine and to reduce animal numbers in pre-clinical studies supporting the 3R concept in animal research and subsequently the numbers of unsuccessful clinical studies and facilitate higher efficiency in the development of new pharmaceuticals and their final application in patients.

P331 Impact Evaluation of Various IVC Housing Systems on Mouse Phenotyping Studies

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With the growing use of genetically engineered mice, a wealth of functional genetic information is being generated. In order to generate consistent data within and across labs, mouse phenotyping studies require standardization of protocols and husbandry conditions. Housing conditions is well known to influence mouse phenotype such as behavior. Individually Ventilated Cage (IVC) housing is becoming popular among animal facilities as this caging system is very effective for animal bioconfinement and improves microenvironemental conditions. Nowadays, various IVC systems with their own specific features are commercially available. While several studies have been done to compare IVC with conventional housing, no information is available regarding how different IVC system may influence mouse phenotypes. We investigated the impact of 3 different IVC housing systems on mouse phenotypes with a systemic phenotyping screen, using the European standard workflow EMPReSSslim (http://empress.har.mrc.ac.uk/). Our results show that most parameters recorded were not affected by the caging system. Nevertheless, some differences were observed depending on the caging system used. Such effects are not likely to occlude potential phenotypes associated with genetic manipulations. These results also argues that phenotyping data obtained under standardized protocol using various existing IVC housing system may be confidently shared or replicated across or within laboratories. This is particularly important for the present and ongoing integrated international phenotyping programs designed to better understand the functions of the genome.

P332 Tibia Bone Mineral Densitometry (BMD) in Different Animal Models of Various Ages: Preliminary Data

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The evaluation of both qualitative and quantitative osteointegration is essential to determine bone scaffolds ability to repair defects and its real biofunctionality. Bone densitometry is considered as a good tool for noninvasively monitoring of healing process. In literature, there are few works about bone density differences between human and most employed animal models, and also on differences between animals with various skeletal development. The aim of this study was to collect normal bone mineral density (BMD) values about tibia in different animal models, because tibia was regarded as the most common experimental implant site. Bone densitometry was detected on three different region of interests (ROI, proximal metaphysis R1, tibia crest R2 and diaphysis R3) in animals of different ages: sheep of < 18 and > 18 months, rabbits of 4, 7 and 12 months, swine of 4, 7 and 36 months, minipigs of 20 months and rats of 2 months. All the data were then compared with normal human's tibia and jaw BMD values. Compared to human's proximal tibia BMD, R1 values were closest in sheep, 4 months swine and minipigs; R2 values were similar with sheep, 7 months swine and minipigs. With regards to human's tibia diaphysis BMD, R3 values were closest in 4 and 7 months swine and in minipigs. Comparing R3 with human's jaw BMD, the middle values was similar to 4, 7 and 12 months rabbits and minipigs. The swine seems to be the most suitable animal model; particularly minipigs, with completed skeletal development, are similar to all considered human's ROI.

P333 Approaching Animal Welfare from a Global Corporate Perspective

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Approaching Animal Welfare from a Global Corporate Perspective. There is no one formula for assuring animal welfare in a global research company. This lecture will discuss a multifaceted program aimed to advance animal welfare efforts not only inside the company, but through industry outreach as well. The Program, the Humane Care Initiative (HCI), was built on the concepts of: employee training, commitment and empowerment; reinforcement; and recognition of excellence. Overall, the program was designed to bring animal welfare to the forefront activities every day of employment and during any interaction with the company. All staff, regardless of job description, receive initial training that includes the company commitment to animal welfare. After this training, the employee signs a statement confirming their alignment with that philosophy. If a person's position involves interaction with animals, initial training is followed by additional training covering the Culture of Caring concept as well as Annual Animal Welfare training on subjects ranging from reporting expectations to animal behavior. Topics change every year but materials are archived on a readily available intranet for use at any time. Conclusions & program implications While there is no one formula for assuring animal welfare, the ideas of initial commitment, consistent reinforcement, recognition and a clear alternatives initiative both inside and outside of a company may be concepts that are universally applicable in the continued commitment to animal welfare in biomedical research.

P334 Multioocyte Follicles (MOFs) in Juvenile C57Bl/6 Mice: A Case Report

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Polyovular follicles or ovarian follicles with more than one oocyte have been observed in immature members of numerous species, including mice. The presence of follicles that contain multiples oocytes (MOFs) is a phenomenonrarely seen in normal laboratory strains. Several signaling pathways such as Notch, estrogen, progesterone and TGF-β have been related to MOFs formation and a sharp decrease in maternal hormonal supply at birth contributes to MOFs formation too. Here we reported a histologic study of follicle development in 21 days and 24 days C57Bl/6 mice counting the follicles at different stages of development. As expected, 24 days animals showed reduced number of primordial follicles, maintenance of primary, secondary and tertiary follicles, and an a slight increase in antral and graafian follicles compared with 21 days mice. Serendipity, 77.7% of animals (7/9) showed MOFs, with the presence of follicles with 2 or 3 oocytes. These results contribute to new information on MOFs presence in laboratory mice.

P335 Orthotopic Transplantation of Mouse Ovaries: Sorted Out and Application Problems

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Orthotopic transplantation of mouse ovarian is a useful technique for managing sub fertile animal colonies to rescue the germ line of mutant or transgenic female mice that are unable to breed or breed poorly, as well as to recover cryopreserved mouse strains from frozen ovaries. In some cases, the animals are extremely fragile or they cannot sustain a productive pregnancy. The unique requisite is the production of functional gametes in donor ovaries. According to the literature, it is expected a fertility reestablishment in between 33 to 75% in transplanted females. Here we reported the sorted out of ovary transplantation technology using C57Bl/6 animals, resulting into a 38.5% of fertility recovery at 30 days after transplantation. Moreover, we attempted to rescue a line of spontaneous obese mutant B6129S mice but, after ovarian transplantation, receptor females failed in to get pregnant after crossing with fertile males either in natural mating or under superovulation induction. Once proved the inability of receptor females to get pregnant, animals were euthanized and ovary tissue was analyzed by histologic techniques.

P336 Mice Colonies Management: Procedures to Ensure Viability

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Successful management of genetically modified mice colonies in biomedical research is an essential point to ensure the efficiency of experimental process. Since the appearance of classic techniques to obtain GM mice, their use have increased to become an essential part of most scientific process, from basic biologic research to applied protocols in pharmaceutical industry (research, development, quality control of product, …). Researchers daily generate new models of genetically modified animals and confront the work to obtain an optimal equilibrium among: - Having a fertile colony - Breeding an appropriate number of mice to fit with experimental procedures. - Breeding a correct mouse model to suit experimental procedures. During the last 6-years period we've managed a total amount of 89 mice colonies with the following targets: planned production of mice for experimental procedures; maintenance of inactive models; backcrossing and transfer of genetic background; new models from crosses of other modified lines. Through the experience gained in these varied managements we are able to design and offer a taylor-made protocol to ensure the quality and efficiency of husbandry for each GM model. This protocol covers all the habitual key points and introduces a tool to confront the occurring events during the initial steps of maintenance. The main targets of this protocol deals with: - Information on fertility, generation, litter size, sexual maturity, reproduction-affecting phenotype, … - Colony optimum dimensioning. - Specific breeding schemes according to genotype, gender o production needs. - Preservation of the appropriate background through generations. - Information management and conservation.

P337 Presentation of the Research Group AWSHEL-IAS (Animal Welfare: Science, Humanities, Ethics and Law. Interdisciplinary Animal Studies)

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The AWSHEL-IAS Research Group originated as a result of the research carried out by University of Alcalá (Madrid, Spain) and the Franklin Institute (a research institute for American studies of the University of Alcalá), for a study on the origin and evolution of the applied science of Laboratory Animal Welfare on both sides of the Atlantic that the scholars 'critical research has been consolidated, hence demanding the need to officially establish the group. The research was extended to other fields of Animal Welfare and new European and American institutions were included. We carry out comparative research on relationships between human and nonhuman animals in the US and Europe. The interdisciplinary approach is maintained by applying theoretical and critical frameworks that include: * Laboratory Animal Sciences: applied Animal Welfare science, neuroscience, ethology and animal cognition. * Ethics: application of moral norms and ethical exegesis of human conduct both at an individual and collective level regarding relationships between humans and nonhuman animals. * Animal Welfare legislation; the legal status of laboratory animals as well as transgenic, wild and

other animals; case-specific legislation regarding animals in research centers or other such as farms, shelters, pet shops, shows and spectacles related to the entertainment industry, fairs, and zoos; and conditions of the commercialization and transport. * Humanities: literature, painting, theatre/cinema and other. It has been thanks to the financial aid of the Franklin Institute. Our next step is to find interaction with European and American institution interested on these issues. If you are interested pease get in touch on cea@uah.es.

P338 A Congenital Cleft Palate Canine Model for Surgical Research Purposes

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The Old Spanish Pointer dog, with a 15% to 20% spontaneous congenital cleft palate rate, is a unique experimental model of this disease. The present study aimed to describe the cleft palate of these dogs for surgical research purposes and to determine whether the congenital cleft palate influences maxillofacial growth. Seven newborn Old Spanish Pointer dogs of both sexes, comprising a cleft palate group (n = 4) and a normal palate group (n = 3), were fed using the same technique. Macroscopic photographs and plaster casts from the palate, lateral radiographs and computer tomographies of the skull were sequentially taken over 41 weeks, starting at week 5. The cleft morphology, the size and the tissue characteristics in these dogs resembled the human cleft better than current available animal models. During growth, the cleft width varies. Most of the transverse and longitudinal measures of the palate were statistically lower in the cleft palate group. The cleft palate group showed hypoplasia of the naso-maxillary complex. This model of congenital cleft palate seems suitable for surgical research purposes. A reduced maxillofacial pre- and post-natal development is associated to the congenital cleft palate presented by the Old Spanish Pointer dog.

P339 Repair of the Injuries of the Cuff Rotator in a Experimental Model with Tipe I Collagen Membrane in Comparison with the Conventional Surgical Procedure with Suture

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To develop a rat model of chronic rotator cuff disease to explore the possible benefits over time of a treatment based on type I collagen membrane compared to the common surgical procedure (suture). A chronic rotator cuff tear injury model was developed by unilaterally detaching the SE tendon of aged Sprague–Dawley rats (9 months). One month post-injury, a second surgery was then performed aimed to repair tears by: a) Classic surgery using single suture (n = 30); b) suture + type I collagen membranes; (n = 30). Lesion restoration was evaluated by biomechanical, histologic criteries at 1 month (n = 20), 2 months (n = 20) and three months (n = 20) post injury All experimental conditions were well tolerated and no adverse effects were observed. Implantation of collagen type I membrane into surgically created tendon defects improved strength, stiffness and deformation of SE tendon three months after treatment compared to the other surgery startegies. None of the treatment achieved an histologic organized pattern. Overall, our findings demonstrate the safety of the treatment used and suggest the therapeutic use of type I collagen membrane in the rotator cuff tears as the most promising treatment for long term. Nevertheless, an organized pattern of fibers was not achieved. Due to the poor organization of collagen fibers, questions concerning the optimal healing time and the type of matrix need to be addressed in future studies. This knowledge ultimately will allow a more appropriate regeneration of the SE tendon.

P340 A Refined Sensitive Venous Bleeding Model for the Assessment of Procoagulant Treatments in Mice

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Haemostatic capacity assessment is a basic tool in development of new targeted treatment of coagulation related diseases therapies, such as coagulation factors supply, homeostasis maintenance involved proteins, new pro/anti-coagulating agents or platelet analogous. Among the most often used tests to account for the haemostatic capacity are either the bleeding time (BT) or the blood loss (BL). In rodents, BT and BL analysis are usually performed by a transection tail tip cut. However, the doses of pro-coagulant treatments for normalizing bleeding in some of these tests are reported to be relatively high. The aim of this study was to refine and simplify an existing sensitive venous bleeding model in FVIII Knock out (F8-KO) mice. We compared the effect of three procoagulant treatments at low dose (lower than 5UI/kg), with two different time approaches (30 compared with 10 minutes) of an innovative saphenous vein injury bleeding model (SBM30; SBM10). We assessed the number of clots formation (NCF), average of bleeding time (ABT), maximum bleeding time (MBT), average blood lost (ABL) and total blood lost (TBL). SBM10 bleeding model represents a cost-effective, time-saving and animal-optimizing protocol compared with SBM30 bleeding model. In the other hand, we found that SBM10 bleeding model allows the assessment of procoagulant treatments efficacy studies as well as SBM30 bleeding model does. All the variables analyzed within the SBM10, showed significant differences between treated and control animals. The present study adds a new refined model to the armamentarium of bleeding models used for the assessment of pro-coagulant compounds.

P341 Model for the Production and Analysis of Prevalence of Antibodies Encephalomyelitis Virus of Theiler

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The Health Monitoring Program for Laboratory Animal implemented by the management of Quality Assurance in CENPALAB, listed for each species a number of microorganisms to diagnose production colonies. Encephalomyelitis virus diseasemouse or Theiler (TMEV) is one of the most studied in this laboratory animal species, because of the degree of prevalence that develops once introduced into breeding colonies. The aim of study was to analyze the prevalence of antibodies against the virus of the Theiler´s encephalomyelitis in BALB/c, experimentally infected. Materials and Methods: To determine the parameters were used to evaluate the methods of indirect ELISA and IFA type. As Positive Control Serum samples 90 mice, BALB/c. (SPF), experimentally inoculated with the virus TEMV, cepa GDVI. The criteria used to determine reading Cut Off was in sum the OD values of the negative control sera and the half of it add up the value of the Negative Control Serum. As evaluation method using a contingency table identifying four boxes: Sensitivity, PPV, NPV, specificity and efficiency using equations established in this connection and statistical methods. Results and

Discussion: The analysis showed that the prevalence was 73 established and 67%, where the values of sensitivity, specificity and kappa of 100%, 76.2% and 0.8 respectively. Conclusions: The ELISA and IFA techniques of indirect type showed high levels of sensitivity and specificity for use en the Control Program Theiler Virus Serology in mouse colonies.

P342 Microbiologic Standards for Mice and Rats Use How Experimentation Animals in Cuba

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Experimentation animals with a defined, controlled, microbiological and genetic quality are essential to achieve reliable results in scientific research. Starting from the study of existing regulations, the route of obtaining, the breeding conditions, the use of animals, the specific pathogenic microorganism and the prevalence of a specific microbiota for experimental rodents, it was designed, defined and evaluated a microbiological standard for mice and rats obtained under controlled conditions at the National Center for Laboratory Animal Breeding (CENPALAB), Cuba. It was carried out an analysis of the microbiological behavior of 11626 rodents for a period of over 20 years, with the aim of determining the prevalence of microbiological entities and their trends using regression analysis. The application of the standard, with the development of a methodology for microbiological monitoring allowed to know that the most prevalent microorganism were four bacteria, two parasites and a murine virus, where the prevalence trend was decreasing over time. The higher prevalence of some microbiological entities was found in animals kept in protected areas. Other entities appeared as microbial contaminants in an accidental or spontaneous way, becoming part of the microbiota resident within them. Applying gnotobiotic techniques and the proper application of good manufacturing practices allow inserting Cuba among the countries with a microbiological standard for the production of rodents free from specific pathogens, ensuring the hygienic quality of these specialized productions highly demanded as experimental biomodels used in Cuban scientific practice.

P343 Respiratory Syndrome Rabbit in Experimental Animals: Prevention

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Pasteurella multocida is considered a common pathogen in rabbits, rabbit farms responsible Respiratory Syndrome (CRS), this may be latent in their host, and the appearance of stress trigger an infection or septic respiratory type, who can act alone or associated with other bacteria such as *Staphylococcus aureus* and *Bordetella bronchiseptica*. The objective of this work is to show the SRC-related microorganisms in a colony of animals for experimentation and evaluation of a vaccine candidate against *Pasteurella multocida*. Six-hundred rabbits were processed CENPALAB, which were taken, samples or nasal swabs trachea and lung, which were processed for culture and identification, isolates were identified with API systems, the candidate vaccine was obtained from a strain of Inactivated field (Bacterin). The results of the study showed that the incidence was higher entity was *Pasteurella multocida* (9.5%), isolated in pure culture, showing their relationship to the respiratory processes of the study population, but also other bacteria were isolated but are associated with respiratory processes this species for experimental animals kept in open (conventional category), does not invalidate its category, these were *Pseudomonas aeruginosa* (7.9%) and Staphylococcus. aureus (7.5%), the preliminary assessment of the candidate vaccine was shown to be safe in the 24 rabbits tested with 3 vaccine doses, being 0.5 mL dose which showed higher optical density values, be concluded that the preliminary results of the vaccine candidate, could be valued as an option, *Pasteurella multocida* bacterin in preventing infections in this species.

P344 Good Practices Guide for the Care, Use and Reproduction of Laboratory Animals in Cuba

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Obtaining and reproduction of experimental animals, use, protection, welfare, microbiological categories for the same and the establishment of ethical principles in experimentation, have been national and international regulations. In order to establish and standardize technical specifications related to these aspects was made the Guide to Good Practice for the care, use and reproduction of experimentation animals (BPCURAL). This document can be using as a national reference for verificating the performance of the Good Clinical Practice and nonclinical and activity of the Committee Institutional Care and Use of Laboratory Animals (IACUC) in the areas of installation, maintenance, care and use of animals, as well as, for training and staff development related. The guide of BPCURAL contain the following headings: Introduction, Definitions and Concepts, Reach and Responsibilities, Basic Principles, and specific ethical animal experimentation, Institutional Program for the Care and Use and Reproduction Laboratory Animal and Annexes and references bibliographic. This guide and manual is part of the quality management system for these experimental biomodels, allowing performing these tasks in the animal facility, vivariums and institutions that use animals in experiments, the same answer as established with the regulations, standards and national and international codes, making it applicable to all staff and structures, because of their work, are directly or indirectly linked to the performance of tasks for the breeding and use of experimental animals. Also will harmonize the principles and criteria used in the national system of experimental animals in Cuba, favoring the transparency in the process of inspections and accreditations.

P345 Preclinical Studies for the Application of Combined Therapy G-CSF and Neuro EPO for Treating Ischemic Cerebrovascular Disease in Its Acute Phase

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Ischemic cerebrovascular disease is the third cause of death in Cuba and the second worldwide. Based in this, the objective of this study was to evaluate the neuroprotector effect of the combined therapy with Neuro EPO and G-CSF in a model of ischemia in the Mongolian gerbil. Neuro EPO and G-CSF were the molecules evaluated.150 animals were used and 6 experimental groups. According to the groups, G-CSF were administered: dose I (10 mg/kg) and dose II (100 mg/kg), both with a single dose (subcutaneously), daily for 7 days and, a single dose of Neuro EPO (5 µL), nasally 3 times a day for 4 days. Mortality and the neurologic status were checked at 24 hours of having carried out the surgery. As behavioral test, was assessed the open field test. The animal survival was better in those animals that received the combined therapy. The neurologic state was same in all the experimental groups. Likewise, there were not differences between the animals that received the combined therapy and those that only received Neuro EPO in the behavioral test. However, there were differences between the animals that received some treatment and those that did not receive any. The G-CSF application possesses a synergistic neuroprotector effect on the Neuro-EPO neuroprotector activity in the acute phase of the cerebral ischemia in the IUP pattern in the Mongolian gerbil.