

Final Program

8th European Congress of Toxicologic Pathology

***Respiratory System Toxicopathology &
Biopharmaceuticals – a challenge for toxicopathology***



***28th September – 1st October 2010
Budapest, Hungary***

organized under the auspices of the
European Society of Toxicologic Pathology



www.eurotoxpath.org

8th European Congress of Toxicologic Pathology
28th September – 1st October 2010 – Budapest, Hungary

Thank You to our Sponsors



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Welcome

Dear Colleagues, Friends, and Guests,

On behalf of the European Society of Toxicologic Pathology (ESTP) and the Hungarian Society of Toxicologists, It is our pleasure to welcome you to the 8th EUROPEAN CONGRESS OF TOXICOLOGIC PATHOLOGY in Budapest, Hungary.

The congress committees (Scientific and Local) have planned an excellent week of sessions on Respiratory System Toxicopathology & Biopharmaceuticals – a challenge for toxicopathology. We encourage you to take a few minutes to look through the schedule of scientific and poster sessions, and special events in this Program.

The “Respiratory System Toxicopathology” sessions include fundamentals of respiratory system toxicology and pathology; animal models and techniques used in research and drug development; pulmonary responses to inhaled nanostructures; and development of inhaled drugs for use in children.

“Biopharmaceuticals – a challenge for toxicopathology” sessions include a wide range of lectures on biopharmaceuticals; issues and challenges in preclinical and clinical safety testing; biodistribution, and pathology of antibodies, stem cells, and vaccines.

The interactive session (which is introduced this year) will take place on the afternoon of 29th September. Interesting and challenging cases will be presented for discussion in this session between our speakers and colleagues, to learn and exchange knowledge from our experiences, and most importantly, to have a good time. Please remember to collect your voting key pads before you enter the congress room.

We have organised some memorable evening activities for you – there is a city bus tour followed by a welcome reception on 28th September; and the Congress Dinner on board the “Europa” (the biggest event boat in Central Europe) on the 30th September. We will have a chance to explore Hungary’s glorious capital Budapest, which possesses a rich and fascinating history as well as a vibrant cultural heritage. Recognizing the unique value of its traditions it has managed to maintain its magic and charm, and is rightly known as the Queen of the River Danube. Side by side you will find the remains of fortresses and buildings from Roman times, Turkish baths, Gothic and Baroque buildings, and the incredibly rich Art Nouveau architectural heritage.

The exhibitors which offer products and services relating to toxicologic pathology are very important part of our meeting. Please stop by their booths during the congress.

Please don’t miss the ESTP Annual Assembly. It will take place on the evening of Wednesday 29th September, when there will be discussion of the pathology peer-review process and the activities of ESTP will be presented to you.

We very much look forward to meeting you during the week, and hope that you enjoy both the meeting and Budapest.

On behalf of the scientific and local organising committees

Sincerely

Zuhal Dincer (Chairman of Scientific Organising Committee)

&

Dezső Dányi (Chairman of Local Organising Committee)



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General Information

Local Organizing Committee

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Catherine Botteron, Ricerca Biosciences, France

Gábor von Bölcsházy, Procter & Gamble Service GmbH, Germany

Ian Taylor, Huntingdon Life Sciences, United Kingdom

Matthias Rinke, Bayer Schering Pharma AG, Germany

Mihály Albert, EGIS Pharma, Hungary

Paul-Georg Germann, Nycomed, Germany

Vasanthi Mowat, Huntingdon Life Sciences, United Kingdom



General Information

Congress Venue

Danubius Health Spa Resort Helia
Kárpát utca 62-64
1133 Budapest
Hungary
Phone: +36-1-889-5800
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Accessibility for Persons with Disability

Please use the front door.

Currency

Hungarian Forint (HUF) is the official currency in Hungary. Currency can be exchanged at hotels, banks, post offices, bureaux de change, airports, railway stations, travel agencies and some restaurants throughout the country. Automatic exchange machines are available in Budapest and other main tourist centres. Currency conversion rates as of Sept 14, 2010: € 1.00 = 284.00 HUF

Please make sure to bring local currencies with you.
Handling of cash machines might sometimes be difficult.

Access to the congress venue from Airport

Please use the Airport Minibus Shuttle Service from the Ferihegy Airport to the City Centre.

Single price to centre: about € 11,00 (2990 HuF)
Single price to airport: about € 18,00 (4990 HuF)

If more people travel to the same address prices are lower.

For more information about the Public Transportation System of Budapest and the timetable of buses see: www.bkv.hu.

Taxi: it is recommended to take a ZONA TAXI. This is a fixed price passenger carrier service; maximized tariffs and fares defined by zones of Budapest.

- Zona Taxi from the airport to the meeting venue (for max 4 pax) € 24,00.
- Zona Taxi from the meeting venue to the airport (for max 4 pax) € 18,00.

Distances from the congress venue to:

International Airport Ferihegy: 26 km
City Centre: 4 km
Eastern (Keleti) Railway Station: 9 km

Parking

Parking lot: € 12/car/day.
The parking place is a closed area, but it is not guarded.

General Information

Climate

Hungary has a temperate continental climate. The summers are dry and warm. Autumns are cool, foggy and rainy. October sees a period which is known locally as the *vénasszonyok nyara* which translates directly to mean 'old woman's summer,' also known as Indian summer. This is due to the continued sunny, clear days which are present throughout September and into October. September sees an average temperature of about 22 °C, before falling to 16 °C in October and falling again to 8 °C by November. Early autumn is a great time to visit Budapest as the summer tourist peak has died down yet the weather is still very enjoyable and there is still an abundance of sunny days.

Registration Desk

The desk will be located at the first floor. All the congress documents can be picked up from the registration desk. An identification badge must be worn to enter all the congress sessions and events. Registration is possible during the whole congress.

Opening hours of registration desk:

28 th September 2010	11:00 h – 18:00 h
29 th September 2010	08:30 h – 17:00 h
30 th September 2010	08:30 h – 17:00 h
1 st October 2010	08:30 h – 13:00 h

The registration area is kindly provided by



Speaker Information

Video beamer and PC are available for presentations. Please turn in your presentations at the front desk before your session. Please use CD-ROM, USB stick or comparable format. The use of your own PC is not desired.

Poster Presentation

Posters will be exhibited during the entire Congress. Poster Sessions are scheduled for the lunch breaks.

An additional poster session will be held on September 30th at 16.30 – 17.30 with Hungarian wine & snacks.

Authors therefore are kindly requested to be at their posters during the lunch break time to answer eventual questions. The poster area is kindly provided by



Language

The official language of the congress will be English. No simultaneous translation will be provided.

Internet Access

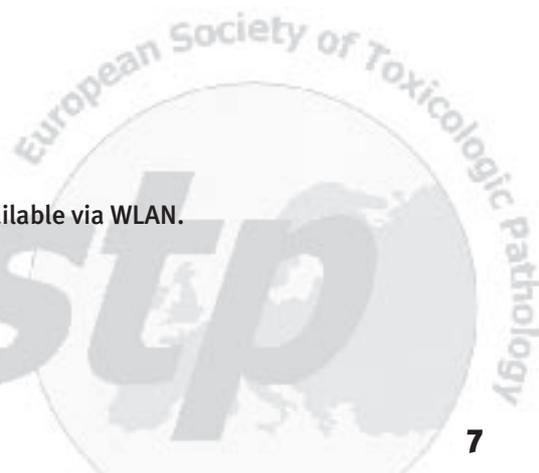
A laptop with internet access is provided for service during the business hours.

The internet access is kindly provided by



In case you want to use your own computer, wireless Internet access is also available via WLAN.

30 min	1.000 Ft = 3,60 €	24 hours	4.000 Ft = 14,40 €
100 min	3.750 Ft = 13,40 €	7 days	10.000 Ft = 36,00 €
2 hours	2.000 Ft = 7,20 €	Prices are including VAT!	



General Information

Messages

There is a message board close to the Congress Registration Desk.

Congress Bags

Congress bags were kindly provided by LPT



Gastronomy

Coffee, tea, refreshment beverage and pastries are served during the coffee breaks

Lunch is provided during the lunch breaks on:

Tuesday, September 28

Wednesday, September 29

Thursday, September 30

Friday, October 1 (only coffee break)

Safety and Security

Please, wear your name badge while in the congress area (access will be denied otherwise)

Remove your name badge when leaving the congress area

Congress representatives will respond to any media inquiries.

In case of emergency please follow directions from the congress staff and chair persons.

Emergency Calls

Police – Emergency 107

Fire – Emergency 105

Ambulance – Emergency 104

ESTP Board Meeting

The meeting will be held on Wednesday, September 29 from 08:00 h – 09:00 h in the Hotel Helia breakfast restaurant.

ESTP Guideline Committee

The meeting will be held on Wednesday, September 29 during the lunch break.

Please ask for the name of the meeting room at the congress counter.

ESTP General Assembly

The annual ESTP General Assembly will be held on Wednesday, September 29 2010 from 17:00 h – 19:00 h in the main session room.

SOC Meeting for the ESTP/ESVP 2011 congress

The meeting will be held on Thursday, September 30 from 08:00 – 08:45 h in the Hotel Helia breakfast restaurant.

General Information

IFSTP Meeting

The meeting will be held on Tuesday, September 28 from 08:30 h – 11:30 h.
Please ask for the name of the meeting room at the congress counter.

IATP Meeting

The meeting will be held on Wednesday, September 29 from 15:30 h – 16:30 h.
Please ask for the name of the meeting room at the congress counter.

ESTP Slide Seminar

An internet slide seminar on different cases of toxicologic pathology is again organized in advance (sponsored by 3DHISTECH KFT.). Case descriptions and scanned slides are available electronically via the ESTP Website www.eurotox-path.org. The contributors will give presentations of their cases during the congress.

Congress CD-ROM

A CD-ROM containing several presentations given at this congress in pdf-format is planned to be handed out to the participants.

The production of the CD-ROM is kindly sponsored by Nycomed GmbH.



Abstract Publication

Abstracts of the presentations and posters will be published in the official journal of the ESTP: Experimental and Toxicologic Pathology in 2011.

Awards

1. SFTP Best Poster Award



2. BSTP sponsored Gopi Award



3. Boehringer Ingelheim ESTP 2010 Award for Scientific Publication

Every two years, the ESTP offers an award for an outstanding thesis in the field of Toxicologic Pathology sponsored by Boehringer Ingelheim Pharma GmbH & Co. KG. The best thesis will be honored with 5.000 Euro, the second best by 3.000 Euro and the third best by 2.000 Euro.



The award ceremony is scheduled for Thursday 30th of September 14:45 – 15:00 h. Please, participate.



General Information

Social Events

September 28th at 17.30

* City bus tour & welcome reception after the presentations

On the first day of the congress, we will get a flavor of Hungary's glorious capital city Budapest. This will be followed by a welcome reception in the congress hotel which is kindly provided by



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September 30th at 16.30 – 17.30

* Poster session with Hungarian wine & snacks

September 30th at 18.30

* For Conference dinner we would like to invite you to a memorable evening with us on board of the “Europa”, the biggest event boat in Central Europe. During the whole evening you can enjoy the magnificent views of Budapest and its surroundings on the River Danube and the famous buildings on either side of the river. The boat departs in front of the hotel and arrives there again after the dinner.

The attire for the welcome reception and the dinner is business casual.

Industry Exhibition

As in previous years, an exhibition featuring Pharmaceutical and Product Companies, Technical Equipment Companies and Medical Publishers will be held within the same setting as the conference. The entrance is free to those registered to the Conference and registered accompanying persons. The exhibition will open on Wednesday, September 29, at 10:30 h and will then follow the same schedule as the conference. At September 30 the exhibition will close after the afternoon coffee break.

The industry exhibition provides information about the newest technologies and developments available within our scientific area. The exhibiting companies have a unique possibility to efficiently reach their target customer. The ESTP values the support from exhibitors and believes that the on-site discussion and exchange of experience between exhibitors and the congress participants is of invaluable importance and benefit.

Exhibition quiz

An exhibition quiz will be performed. The documents needed for your participation will be handed out to you at the congress counter. The 1st prize is the book “Fundamentals of Toxicologic Pathology, 2nd Edition“ (By Wanda M. Haschek, Matthew A. Wallig and Colin G. Rousseaux), and the winner will be awarded on Thursday during the afternoon coffee break.

The book is kindly provided by



Please, visit the booths of our exhibitors.

General Information

Thanks to our Exhibitors

The ESTP greatly values the support from the following Exhibitors

Aperio Technologies	www.aperio.com
ELP	www.epl-inc.com
Hamamatsu	www.hamamatsu.com
3D Histech	www.3dhistech.com
Instem	www.instem-lss.com
PDS	www.pds-europe.com
Slidepath	www.slidepath.com
Visiopharm	www.visiopharm.com
Xybion	www.xybion.com

General Information

Thanks to our Sponsors

The ESTP greatly values the support from the following Sponsors

Aperio Technologies	www.aperio.com
Astra Zeneca R&D	www.astrazeneca.com
BASF	www.basf.com
BSTP	www.bstp.org.uk
Elsevier	www.elsevier.com
GlaxoSmithKline	www.gsk.com
3D Histech	www.3dhitech.com
Instem	www.instem-lss.com
Johnson & Johnson	www.jnj.com
LAB	www.labresearch.com
LPT	www.lpt-pharm-tox.de
Novartis	www.novartis.ch
Nycomed	www.nycomed.com
Novo Nordisk	www.novonordisk.com
Pathology Experts	www.pathexperts.com
PDS	www.pds-europe.com
Pfizer	www.pfizer.com
SFPT	www.toxpathfrance.org
Visiopharm	www.visiopharm.com



Congress Program

28th September, Tuesday

11.00 – 13.30 **Registration & Lunch**

13.30 – 13.40 **Welcome**

Ingrid Sjögren, ESTP president (Novo Nordisk, Denmark)
Dezső Dányi, Chairman of LOC (Gedeon Richter Plc, Hungary)

Respiratory System Toxicopathology

13.40 – 13.45 **Introduction**

Zuhal Dincer, Chairman of SOC (Pfizer, United Kingdom)

Session Co-Chair: Vasanthi Mowat (Huntingdon Life Sciences, United Kingdom)

13.45 – 14.30 **S 01: Preclinical safety assessment for inhaled drugs**

Keith Owen (Pfizer, United Kingdom)

14.30 – 15.00 **S 02: Infectious diseases of Respiratory System**

Charles Clifford (Charles River Laboratories, USA)

15.00 – 15.30 **Coffee Break**

15.30 – 16.15 **S 03: Animal models of asthma & COPD**

Sarah Bolton (AstraZeneca, United Kingdom)

16.15 – 17.00 **S 04: Development of inhaled pharmaceuticals for children – a pathologist's perspective**

Jan Klapwijk (GlaxoSmithKline, United Kingdom)

17.30 – 19.30 **City Bus Tour**

19.30 – 20.30 **Welcome reception in the congress hotel**
is kindly provided by



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Congress Program

29th September, Wednesday

Respiratory System Toxicopathology continues

Session Co-Chair: Gábor von Bölcsházy (Procter & Gamble Service GmbH, Germany)

- 09.00 – 09.45 **S 05: Lung histopathology of animal models used in research and drug development**
Paul-Georg Germann (Nycomed, Germany)
- 09.45 – 10.30 **S 06: Invasive and non-invasive lung function measurements in rodents in toxicological research and drug safety**
Holger Schulz (Institute of Lung Biology & Disease, Germany)
- 10.30 – 11.00 **Coffee Break**
- 11.00 – 11.45 **S 07: The respiratory tract as portal of entry for inhaled nanoparticles**
Günter Oberdörster (University of Rochester, NY, USA)
- 11.45 – 12.15 **S 08: Application of special labelling techniques (IHC, ISH) in the respiratory tract**
Richard Haworth (GlaxoSmithKline, United Kingdom)
- 12.15 – 13.30 **Lunch**
- Session Co-Chair: Tom Brodie (Pfizer, United Kingdom)*
(interactive session)
- 13.30 – 14.00 **S 09: Assessing mechanism of lung cancer**
Robert Sills (NIEHS, USA)
- 14.00 – 14.30 **S 10: Hyperplasia/tumours – upper respiratory system**
Heinrich Ernst (Fraunhofer ITEM, Germany)
- 14.30 – 15.00 **S 11: Hyperplasia/tumours – lower respiratory system**
Susanne Rittinghausen (Fraunhofer ITEM, Germany)
- 15.00 – 15.30 **Coffee Break**

Congress Program

29th September, Wednesday

Session Co-Chair: Catherine Botteron (Ricerca Biosciences, France)
(interactive session)

15.30 – 15.45 **Case presentation 01**
Franck Chanut (GlaxoSmithKline, United Kingdom)

15.45 – 16.00 **Case presentation 02**
JoAnn Schuh (Applied Vet Pathobiology, USA)

16.00 – 16.15 **Case presentation 03**
Ingo Gerhauser (TiHo Hannover, Germany)

16.15 – 16.30 **Case presentation 04**
Francesco Marchesi (Accelerera, Italy)

16.30 – 17.00 **SATELLITE SYMPOSIUM**
Lars Pedersen (Visiopharm, Denmark)
Principles of design-based stereology and its applications to animal models of lung diseases

17.00 – 19.00 **ESTP Annual Assembly**

Congress Program

30th September, Thursday

Biopharmaceuticals – a challenge for toxicopathology

- 08.55 – 09.00 **Introduction**
Zuhal Dincer, Chairman of SOC (Pfizer, United Kingdom)
- Session Co-Chair: Paul-Georg Germann (Nycomed, Germany)*
- 09.00 – 09.45 **S 12: The issues of immunogenicity/immune complex disease to biopharmaceuticals**
Kevin McDorman (Charles River Laboratories, USA)
- 09.45 – 10.30 **S 13: Safety Assessment of Antibodies**
Frank Brennan (Novartis, Switzerland)
- 10.30 – 11.00 **Coffee Break**
- 11.00 – 11.30 **S 14: Species selection in pre-clinical testing of biopharmaceuticals**
Jeffrey Engelhardt (EPL, USA)
- 11.30 – 12.00 **S 15: Nonclinical development of bispecific T cell engaging BiTE[®] antibodies**
Benno Rattel (Micromet, Germany)
- 12.00 – 13.15 **Lunch**
- Session Co-Chair: Matthias Rinke (Bayer, Germany)*
- 13.15 – 14.00 **S 16: Issues and challenges in pre-clinical safety testing of stem cells therapies**
Dominique Brees (Pfizer, United Kingdom)
- 14.00 – 14.45 **S 17: Experiences with FDA & EMEA on biopharmaceuticals**
Lauren Black (Charles River Laboratories, USA)
- 14.45 – 15.00 **Award Ceremony**
- 15.00 – 15.30 **Coffee Break**
- Session Co-Chair: Mihály Albert (EGIS Pharma, Hungary)*
- 15.30 – 16.00 **S 18 Roadmap of a VLP vaccine for Chikungunya virus**
Srinivas Rao (National Institute of Health, USA)
- 16.00 – 16.30 **S 19: Rat Respiratory Virus**
Charles Clifford (Charles River Laboratories, USA)
- 16.30 – 17.30 **Poster Session**
(with Hungarian wine, soft drinks & snacks)
- 18.30 **ESTP Dinner**

Congress Program

1st October, Friday

Biopharmaceuticals – a challenge for toxicopathology continues

Session Co-Chair: Ian Taylor (Huntingdon Life Sciences, United Kingdom)

- 09.00 – 09.45 **S 20: Therapeutic vaccines & adjuvants**
Michaela Sharpe (Pfizer, United Kingdom)
- 09.45 – 10.30 **The BSTP Chirukandath Gopinath Lecture**
S 21: Pathology of injected biopharmaceuticals
Jeffrey Engelhardt (EPL, USA)
- 10.30 – 11.00 **Coffee Break**
- 11.00 – 11.30 **S 22: Cytokine release syndrome associated with therapeutic antibodies**
Frank Brennan (Novartis, Switzerland)
- 11.30 – 12.15 **S 23: Biodistribution of biopharmaceuticals & cross reactivity studies**
Kevin McDorman/Jennifer Rojko (Charles River Laboratories, USA)
- 12.15 – 12.30 **Closing remarks**
Ingrid Sjögren, ESTP Past President (Novo Nordisk, Denmark) & Annette Romeike, ESTP President (IPSAR Consulting, France)



See you in Sweden in 2011

Speaker Abstracts

So1: Preclinical Safety Assessment for Inhaled Drugs

Keith Owen

Drug Safety R&D, Pfizer Global R&D, Sandwich, Kent, CT13 9NJ

To treat respiratory diseases it is desirable to administer drugs via inhalation to achieve direct exposure of the target tissues. This direct exposure promotes a rapid onset of action at the target site and allows for the use of small doses, which limit systemic exposure and consequently reduce the likelihood of undesirable side effects. Systemic exposure can be further limited by designing molecules which are metabolically labile, and have high potency, low aqueous solubility and low oral bioavailability.

Preclinical safety packages should be conducted in accord with international regulatory guidelines, and the repeat dose toxicology studies must utilise the inhaled route in order to characterise both local (respiratory) and systemic toxicity. For other study types (eg reproductive and safety pharmacology studies) it is acceptable to conduct them via intravenous or subcutaneous routes as it is paramount to maximise systemic exposure.

Respiratory tract changes in preclinical species can include irritancy affecting the larynx and/or nasal cavity, particularly in rodents. However, such changes are not necessarily predictive of a risk to humans due to exquisite sensitivity of the rodent larynx and the lack of exposure to the nasal cavity following oro-inhalation of drugs in the clinical setting.

The design of insoluble molecules to limit systemic exposure means that there is a greater emphasis on elimination via macrophages. Consequently, the presence of macrophages in the lungs is often noted and in the absence of any other changes this is generally considered to be a non-adverse, physiological response to an inhaled particulate. Other changes in the lung, such as inflammation and/or epithelial hyperplasia, resulting from irritancy or particulate overload are of concern as they are not monitorable in humans. For such changes, safety margins are calculated in terms of the drug deposited per unit weight of lung. While a human lung deposition factor related to inhaled fine particle mass (usually around 20-40%) is the most scientifically relevant, the FDA assume 100% deposition; they also expect a safety margin of at least 10 fold for rodents and 6 fold for non-rodents.

All of these factors should be taken into account when designing preclinical studies or programmes for inhaled drugs.



Speaker Abstracts

So2: Infectious Diseases of the Respiratory System of Laboratory Rodents

Charles B Clifford, DVM, PhD, DACVP

Charles River Research Animal Diagnostic Services

Although infectious respiratory disease in laboratory rodents is less common now than in the last century, toxicologic pathologists must still occasionally attempt to distinguish study-related effects from lesions due to infectious disease, not to mention a background of non-infectious strain-related lesions. This interactive presentation will attempt to provide some context for those decisions. We will discuss the gross and histologic appearance of infectious respiratory diseases including a few “classical” agents as well as those infectious agents which still occur in SPF research facilities. We will also note which currently prevalent infectious agents are NOT associated with morphologic alterations. In addition, brief mention will be made of the diagnostic tools available to help resolve the question of whether a specific agent played a role in causing respiratory lesions. Specific agents to be discussed include those which cause suppurative inflammation (*Mycoplasma pulmonis*, cilia-associated respiratory bacillus, and *Bordetella* spp.), those which cause non-suppurative inflammation of the airways (rat coronavirus or sialodacryoadenitis virus, and parainfluenza viruses type 1 – 3, including Sendai), and those which cause non-suppurative interstitial pneumonia (the so-called “rat respiratory virus”, pneumonia virus of mice). For each agent, we will also give a rough estimate of current prevalence in North American and European research facilities.

Speaker Abstracts

S03: Animal models of COPD and asthma

Sarah Bolton

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Animal models are frequently used to test candidate drugs and to give confidence that a test compound will work in human disease. However, these animal models are too reductive and will at best, model maybe only one aspect of a human condition and there is a clear disconnect between the disease and the experimental animal paradigm. The differences between human systems and rodents are significant including basic anatomy, differences in cell types and turnover rates as well as the pathological consequences of a particular insult. It is also very difficult to model the complex contributions of both genetic and environmental factors. Respiratory diseases such as chronic obstructive pulmonary disease (COPD) and asthma are also subject to animal modeling using various inhaled challenges such as ovalbumin or lipopolysaccharide, but these are particularly focused on leucocyte infiltration. More clinically relevant challenges such as tobacco smoke or house dust mite are also used but even these animals may not mimic key structural changes seen in the human tissue. Recently, several publications have highlighted the potential of using multi-factorial models or even genetically predisposed rodents such as the spontaneously hypertensive rat. However, in order to get more information from these experiments, greater clarity about what is being modeled and how the target relates to human disease needs to be better understood. Finally, toxicological assessment of candidate drugs in a diseased lung setting also needs to be more fully considered using the animal models.

Speaker Abstracts

So4: Development of inhaled pharmaceuticals for children – a pathologist's perspective

Jan Klapwijk

Director and Head of Pathology, UK Safety Assessment, GSK

The last 10 years has seen the introduction of a number of Regulatory guidelines that require the preclinical testing in juvenile animals of drugs intended for use in children. This presentation will start by introducing these Guidelines.

The lung is an example of an organ in which a great deal of development occurs after birth. An understanding of the normal development and histology of young growing animals is essential in the pathological evaluation of this age group. To this end we are building up a library of tissues from young animals of different ages. Images from this library will be used to illustrate some of the changes occurring as the lung develops post-natally. Species differences and comparison with human will be mentioned, in particular how these impact on age at start of dosing and study endpoints. Other aspects of study design and practicalities associated with dosing, by inhalation, immature / growing animals will also be discussed.

This presentation will also consider factors which may make young growing tissues sensitive to mechanisms of toxicity not seen in adults and the implications for risk assessment.

Speaker Abstracts

S05: Use of histopathology in pharmacological models for respiratory diseases: Case studies for ARDS, Asthma and measles virus pneumonia

Paul-Georg Germann and Andreas Pahl

Nycomed GmbH, Discovery to Preclinical Development, Constance and Institute for Pharmacology & Preclinical Drug Safety, Willinghusen, Germany

Pharmacology models should mimic the human disease pattern as close as possible. This is a key factor for the successful translation of preclinical efficacy into the clinical situation. Using the expertise of investigative pathologists for the set up and the validation of these models and their evaluation criteria adds substantial value to this process. Three examples of respiratory disease models, the experiences and key pitfalls are presented and discussed.

The cotton wool rat, a species susceptible for the human measles virus, is used for studying prophylactic and therapeutic treatments of the measles virus pneumonia. The measles virus induced pneumonia is still one of the most important causes of death in small childrens of developing countries. The time sequence of the histopathological lung events after experimental infection is presented.

The Brown Norway rat, sensitized and challenged with ovalbumin, is widely used for the evaluation of new therapeutic concepts in asthma this model the terminal analysis of the lung and the bronchio-alveolar lavage fluid with cellular and a-cellular readouts is the standard parameter set. Especially histopathology plays an essential role in the validation or also devalidation of this model. The deficiencies of this model can nicely be demonstrated by histopathology.

Thirdly the rat lung lavage model, inducing a disease similar to the exsudative early phase of the acute respiratory distress syndrome (ARDS) in humans is substantially dependant on histopathological evaluation. Examples will be given demonstrating the treatment effects of surfactant and the various experimental dependencies in this model.

In conclusion, the morphological and the functional working scientists should liaise very closely with their colleagues in their respective expertise areas. This improves the quality of the animal model and the obtained results for a better selection of drug candidates. The validation of the model is the key to success.



Speaker Abstracts

So6: Invasive and non-invasive lung function measurements in rodents in toxicological research and drug safety

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With the increasing role of rodents, particularly mice, in toxicological and translational research on the respiratory system, the availability of lung function tests which are easy to implement and allow repetitive and, possibly, non-invasive studies, became a frequent request. Several techniques available for humans or larger experimental animals have been adapted taking up the challenges associated with the small body sizes of rodents. This presentation will introduce basic parameters of lung volumes, respiratory mechanics and gas exchange and describe the corresponding measurement techniques, illustrated by examples from irritant exposure studies and research on restrictive, obstructive and degenerative lung diseases.

For high-throughput studies on conscious subjects in a low-stress environment, unrestrained whole body plethysmography has gained wide popularity. The system is seductive in its simplicity and non-invasiveness – the rodent is placed in a closed chamber and parameters like tidal volume, respiratory rate and timing are derived from cyclic pressure swings generated in the chamber during breathing. The system is often applied to assess airway responsiveness to aerosolized beta agonists by measuring Penh, a dimensionless index derived from respiratory flow and timing. However, this is not a measure of resistance and may substantially be altered with other conditions such as lung edema. Hence, the role of Penh is controversial in the respiratory community and confirmation of results by classical methods is recommended.

For specific assessment of airway resistance and dynamic compliance of the respiratory system, more controlled experimental conditions are required. Rodents are then anesthetized, tracheotomized or intubated, and placed into a box with the cannula connected to the environment. Respiratory system resistance and dynamic compliance are derived from airway opening pressure and flow/volume readings of individual breathing cycles during baseline conditions and subsequent delivery of bronchoconstricting aerosols. Alternatively, the forced oscillation technique is technically demanding and sophisticated, yet it provides the most specific method for the characterization of respiratory mechanics. The respiratory system is challenged with a broad-band volume perturbation signal composed of simultaneous waveforms containing different frequencies. From flow and pressure response signals, the real and imaginary parts – resistance and reactance, respectively – of respiratory system impedance are calculated based on an appropriate mathematical model of lung mechanics. Application of this technique during bronchial challenge allows to distinguish specifically between airway and lung tissue responses.

Airway obstruction in humans is typically identified with forced expiratory maneuvers and measurement of the volume exhaled within one second (FEV₁). To achieve a forced expiration in rodents, high negative pressure is applied to the airway opening of tracheotomized animals following a full lung inflation. During the maneuver, exhaled flow is recorded as a function of the expiration time or volume providing the maximum expiratory flow-volume curve. Forced exhalation flows are physiologically limited due to collapse or compression of airways and are thus a function of lung elastic recoil properties and airway resistance. While flow-volume curves are qualitatively similar in normal lungs of various species, distinct differences have to be considered in diseased lungs, for example, in emphysema.

According to the “Phenotyping Uncertainty Principle”, selection of the most appropriate lung function technique has to find a balance between natural conditions at the cost of precision, specificity and sensitivity, or precise measurements under controlled but artificial conditions. The methods described above provide the basis for researchers to select a suitable measurement technique for their particular purposes and to find a compromise between maintenance of the most natural conditions and achievement of specific, sensitive and precise measurements.

Speaker Abstracts

S07: The Respiratory Tract as Portal of Entry for Inhaled Nanoparticles

Günter Oberdörster

University of Rochester, Rochester, NY, USA

Multiple applications of nanotechnology in diverse industries, consumer products and medicine have raised serious concerns about potential adverse effects following exposure to nanomaterials. Inhalation of airborne nanoparticles (NPs) is a major route of exposure, and knowledge about such exposure as well as about hazard of NPs is necessary to determine if there is a real or only a perceived risk. When inhaled, NPs deposit efficiently in all regions of the respiratory tract by diffusion; however, the smallest NPs (<~10 nm) deposit most efficiently in the upper respiratory tract. A major difference to larger particles is the propensity of NPs to translocate from the site of deposition in the respiratory tract to secondary extrapulmonary organs, involving along neuronal pathways (to the CNS) and across epithelial barriers (*via* blood, lymph, to other secondary organs). However, only a very small fraction of deposited NPs will translocate, and more information about such biokinetic behavior is urgently needed as guide for selecting appropriate doses for designing *in vitro* toxicity assays with NPs. Central concepts of nanotoxicology include consideration of dose, dose-rate, and the importance of detailed physico-chemical characterization of NPs, specifically surface properties, that influence their biological/toxicological properties and their biokinetics. Key is to identify potential effects induced at relevant exposures at the portal of entry, the respiratory tract, and in secondary organs (*e.g.*, neurodegeneration in the CNS from translocated NPs? mesothelioma in the pleural cavity from carbon nanotubes?). Some, but probably not the majority of NPs, will have a significant toxicity (hazard) potential at realistic exposures. One challenge is to identify such hazardous NPs and taking appropriate measures to prevent exposure. The ultimate goal of a meaningful risk assessment requires data about exposure assessment and hazard identification, including information about biopersistence and mechanisms in cells and subcellular structures.



Speaker Abstracts

So8: Application of special labelling techniques (IHC, ISH) in the respiratory tract

Richard Haworth

GlaxoSmithKline, United Kingdom

In toxicological pathology there are a range of situations in which information, additional to that readily observed with conventional haematoxylin and eosin stained sections, needs to be obtained from respiratory tract tissue sections. Examples which will be addressed and illustrated in this presentation include the demonstration of antigen integrity as part of tissue cross reactivity (TCR) studies, demonstration of xenobiotic location, histogenesis of hyperplasia or neoplasia and characterisation of cellular inclusions and cellular infiltrates. Available techniques which will be discussed include specialised histochemistry, immunohistochemistry (IHC) and in-situ hybridisation (ISH). Application of IHC to the nasal cavity can be technically difficult because of the effect of decalcification on antigen integrity. In each situation the simplest technique, e.g. histochemistry, which answers the question should be used first.

Speaker Abstracts

S09: Molecular Assessment of Mouse Lung Tumors and Relevance to Humans in NTP Studies

Robert C. Sills, Arun Pandiri, Hue L. Hong, Thai-Vu T. Ton, Stephanie A. Lahousse and Mark J. Hoenerhoff

Cellular and Molecular Pathology Branch, National Toxicology Program, National Institute of Environmental Health Sciences, Research Triangle Park, NC, USA

In the US National Toxicology Program, the B6C3F1 mouse provides an excellent model for assessing chemically induced lung tumors and their relevance to human lung cancer. The alveolar bronchiolar tumors in B6C3F1 mice are morphologically similar to non-small cell lung tumors in humans. In addition, the altered cancer genes and pathways are comparable within mouse and human lung tumors. The presentation will focus on the molecular assessment of oncogenes and tumor suppressor genes in spontaneous and chemically induced mouse lung tumors and provide data on specific genetic alterations and genome wide assessments of cancer genes in mouse lung tumors. Examples include demonstration of genetic alterations in K-ras and p53 genes by mutation analysis in mouse lung tumors following transplacental AZT exposure; and demonstration by global gene expression and mutation analysis that K-ras mutations and activation of MAP kinase signaling pathway in cumene-induced mouse lung tumors resulted in greater malignant phenotype compared to lung tumors with no K-ras mutations. The presentation will conclude by providing information on current approaches that are being used to obtain a broader perspective on identifying important genetic and epigenetic events in B6C3F1 mouse lung tumors and thereby providing a greater relevance to human lung cancer risk assessment.

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Speaker Abstracts

S10: Proliferative Lesions of the Upper Respiratory Tract in Rodents

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Proliferative lesions in the upper respiratory tract of laboratory rodents rarely develop spontaneously. They may be induced, however, by a wide variety of xenobiotics and irritant chemicals either by inhalation or other routes of exposure. Due to its structural and functional complexity, toxicant-induced proliferative lesions are observed more often in the nasal cavity than in the pharynx, larynx or trachea.

In this interactive session, some of the proliferative lesions listed belowx) will be shown and discussed with consideration of related differential diagnoses. For each of the presented cases which were taken from the species rat, mouse or Syrian hamster, everybody in the audience will be asked to provide his/her diagnosis.

x) Terminology of Proliferative Lesions of the Upper Respiratory Tract in Rats and Mice

I. Nasal Cavity

Non-neoplastic Proliferative Lesions

Metaplasia, Squamous Cell
Metaplasia, Respiratory, Olfactory/Glandular Epithelium
Hyperplasia, Squamous Cell
Hyperplasia, Transitional Epithelium
Hyperplasia, Respiratory Epithelium
Hyperplasia/Metaplasia, Mucous Cell
Hyperplasia, Olfactory Epithelium
Hyperplasia, Basal Cell
Hyperplasia, Neuroendocrine Cell
Hyperplasia with Atypia

Neoplastic Proliferative Lesions

Papilloma, Squamous Cell
Adenoma
Carcinoma, Squamous Cell
Carcinoma, Adenosquamous
Adenocarcinoma
Carcinoma, Neuroepithelial

II. Larynx, Trachea

Non-neoplastic Proliferative Lesions

Epithelial Alteration
Metaplasia, Squamous Cell
Hyperplasia, Squamous Cell
Hyperplasia, Respiratory Epithelium
Hyperplasia, Mucous Cell
Hyperplasia, Neuroendocrine Cell
Hyperplasia with Cellular Atypia (Dysplasia)

Neoplastic Proliferative Lesions

Papilloma
Tumor, Neuroendocrine Cell, Benign
Carcinoma, Squamous Cell
Adenocarcinoma
Tumor, Neuroendocrine Cell, Malignant

Reference

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Speaker Abstracts

S11: Proliferative Lesions of the Lower Respiratory Tract in Rodents

Susanne Rittinghausen

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Proliferative lesions in laboratory rodents may arise from infectious agents or during aging, but most proliferative respiratory tract lesions result from inhalation exposure to potentially toxic test material or particles. Cellular destruction from prolonged exposure to toxicants induces a repair process in which the target tissue may proliferate and/or undergo metaplasia to a different, more resistant cell type, or in the case of genotoxic compounds, gives rise to neoplasia.

In this interactive session several proliferative lesions taken from rats or mice will be shown and discussed with the audience together with related differential diagnoses under consideration of the International Harmonized Nomenclature (International Harmonization of Nomenclature and Diagnostic criteria – INHAND), recently published for the respiratory system by an international organ working group under supervision of STP, ESP, BSTP and JSTP.

Terminology of Proliferative Lesions of the Lower Respiratory Tract in Rats and Mice

Bronchi; Lung, Bronchioles^{*)}

Hyperplasia, mucous cell
Hyperplasia, neuroendocrine cell
Hyperplasia, respiratory epithelium
Hyperplasia, squamous cell
Hyperplasia, with cellular atypia (dysplasia)
Metaplasia, squamous cell

Papilloma

Tumor, neuroendocrine cell, benign
Adenocarcinoma
Carcinoma, squamous cell
Tumor, neuroendocrine cell, malignant

Lung, Terminal Bronchioles^{*)}

Hyperplasia, bronchiolo-alveolar
Metaplasia, mucous cell
Metaplasia, Squamous Cell

Adenoma, bronchiolo-alveolar

Carcinoma, acinar

Carcinoma, adenosquamous
Carcinoma, bronchiolo-alveolar
Carcinoma, squamous cell

Lung, Alveoli^{*)}

Cyst, pulmonary keratinizing
Hyperplasia, bronchiolo-alveolar
Metaplasia, mucous cell
Metaplasia, squamous cell

Adenoma, bronchiolo-alveolar

Epithelioma, cystic, keratinizing
Epithelioma, nonkeratinizing
Carcinoma, acinar
Carcinoma, adenosquamous
Carcinoma, bronchiolo-alveolar
Carcinoma, squamous cell

Pleura

Hyperplasia, mesothelial
Mesothelioma, malignant

Reference

- ^{*)} Renne R, Brix A, Harkema J, Herbert R, Kittel B, Lewis D, March T, Nagano K, Pino M, Rittinghausen S, Rosenbruch M, Tellier P, Wohrmann T.: Proliferative and nonproliferative lesions of the rat and mouse respiratory tract. *Toxicol Pathol.* 2009; 37 (7 Suppl):5S-73S.

Speaker Abstracts

ESTP Satellite Symposium

Principles of Design Based Stereology and Its Application to Animal Models of Lung Diseases

Lars Pedersen

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Earlier this year, the ATS and ERS published a joint task force report titled *An Official Research Policy Statement of the American Thoracic Society/European Respiratory Society: Standards for Quantitative Assessment of Lung Structure* (Hsia, C.C.W. et al., 2010, *Am J Respir Crit Care Med* 181(4):394-418). The report defines new standards for obtaining quantitative measurements of lung structure using design based stereology methods. Recommendations for tissue fixation and tissue sampling are given together with useful lung parenchyma morphometric end points. Topics regarding assessment of cell ultra structure, larger airways and vascular systems, biopsies, and new in-vivo imaging techniques are also covered in the report.

The importance of the ATS/ERS research policy statement cannot be underestimated in the efforts to spread and implement sound, unbiased methodology to the world of lung research. Hopefully, many researchers will soon adopt these methods to ensure accurate quantification of lung structures and easy comparison of results from different studies.

This presentation aims at illustrating how modern computer assisted stereology can help support the recommendations in the ATS/ERS research policy statement. A brief introduction to principles of design based stereology will be given together with illustrations of methods to obtain selected morphometric end points in histological sections of the lung parenchyma as outlined in the ATS/ERS research policy statement using dedicated software for computer assisted stereology. Selected end points will include total lung volume, number of alveoli, alveolar surface area, alveolar mean size, and alveolar septal thickness in histological sections from different animal models; monkey, rat and mouse.

Speaker Abstracts

S12: The Issues of Immunogenicity/Immune Complex Disease in the Development of Biopharmaceuticals

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Development of biopharmaceuticals involving animal test systems may encounter hurdles in the area of immunogenicity and immune complex generation. Immune reactions and immune complex generation do not represent abnormalities per se, they more represent the mounting of an immune response of biological systems to a foreign antigen. The impact of immunogenicity on the pathology assessment and overall risk assessment of biopharmaceuticals may be negligible or dramatic. A low incidence of immunogenicity, particularly if adequately characterized and reasonably determined to represent generation of a normal immune response, may have a negligible impact on a biopharmaceutical development program. But a high incidence of immunogenicity can have a dramatic impact, particularly if neutralizing antibodies have altered anticipated exposure levels or if the nature of the immune response is left undetermined. Characterization of the immune response is important in order to understand the biological and pathological relevance of the effect and provide some idea of translational importance to the target human population. The potential of a novel biopharmaceutical to produce significant immunogenicity and/or immune complex deposition cannot be accurately predicted based on structure or composition. However, the general rule is that the further the species of origin of the biopharmaceutical to the test system or target human population, the greater the risk of immunogenicity and/or immune complex generation. In addition, the translational ability of the incidence of immunogenicity/immune complex generation of the biopharmaceutical in nonclinical test systems to predict the incidence of immunogenicity in the target human population is low. Many unresolved issues must be addressed and understood before effective prediction can become routine.



Speaker Abstracts

S13: Immunopharmacology and Immunotoxicity Testing of Monoclonal Antibodies

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Novartis Biologics, Translational Sciences and Safety

Abstract

The majority of therapeutic monoclonal antibodies (mAbs) licensed for human use or in clinical development are indicated for treatment of patients with cancer and inflammatory/autoimmune disease and as such are designed to directly interact with the immune system. A major hurdle for the development and early clinical investigation of many of these immunomodulatory mAbs is their inherent risk for adverse immune-mediated drug reactions in humans such as infusion reactions, cytokine storms, immunosuppression and autoimmunity. A thorough understanding of the immunopharmacology of a mAb in humans and animals is required to both anticipate the clinical risk of adverse immunotoxicological events and to select a safe starting dose for First-in-Human (FIH) clinical studies. This presentation aims to summarize the most common adverse immunotoxicological events occurring in humans with immunomodulatory mAbs and outlines non-clinical strategies to define their immunopharmacology and assess their immunotoxic potential, as well as to reduce the risk of immunotoxicity through rational mAb design. Tests to assess the relative risk of mAb candidates for cytokine release syndrome, innate immune system (dendritic cell) activation and immunogenicity in humans will also be discussed. The importance of selecting a relevant and sensitive toxicity species for human safety assessment in which the immunopharmacology of the mAb is similar to that expected in humans is highlighted as is the importance of understanding the limitations of the species selected for human safety assessment and supplementation of in vivo safety assessment with appropriate in vitro human assays. A tiered approach to assess effects on immune status, immune function and risk of infection and cancer, governed by the mechanism of action and structural features of the mAb, is advocated. Finally, the use of immunopharmacology and immunotoxicity data in determining a Minimum Anticipated Biologic Effect Level (MABEL) and in the selection of a safe human starting dose will be discussed.

Speaker Abstracts

S14: Species selection in preclinical testing of biopharmaceuticals

Jeffery Engelhardt

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Selection of species for toxicity testing of small molecule therapeutics is generally a simple task as rat and dog are the most common preclinical test subjects. Selecting the appropriate species for toxicity studies with biotherapeutic agents is not always as straightforward. To provide the best characterisation of the safety profile of a candidate biotherapeutic, the animal species utilised in the toxicity studies should be a pharmacologically relevant species. That is, an animal species that possesses the desired biological target and where the target behaves in a similar manner as desired in humans when exposed to the candidate biotherapeutic. Several considerations need to be made when deciding if a species meets the necessary criteria. These considerations include, but are not limited to, protein sequence homology among the species, binding affinity and avidity to the target for monoclonal antibodies, tissue expression and distribution of the target in the various species, activity of the biotherapeutic in isolated cell systems, *ex vivo* bioassays, and *in vivo* pharmacodynamic activity. Immunogenicity should also be considered but should not be used as the only reason to discount a species at the outset of testing. Should only one relevant species be identified, i.e., the cynomolgus monkey, the preclinical safety programme may be conducted in the monkey alone. This strategy is acceptable but needs to be justified in the regulatory documents. Conducting toxicity studies in an irrelevant species is discouraged, as the results cannot be extrapolated for human safety of the candidate biotherapeutic.

Speaker Abstracts

S15: Non-clinical Development of Bispecific T Cell-engaging BiTE Antibodies

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Bispecific antibodies can transiently link tumor cells with resting polyclonal T cells for induction of a surface target antigen-dependent redirected lysis of tumor cells. One example is blinatumomab, which belongs to a class of bispecific biologics called BiTE[®] antibodies (for bispecific T cell engager). *In vitro*, blinatumomab and other BiTE antibodies activate T cells in a highly conditional manner that is dependent on the presence of target cells. Blinatumomab has two covalently linked single-chain antibody variable domains (scFv) directed against CD3 and CD19, respectively. Blinatumomab thereby combines on a single polypeptide chain the binding specificities for both the pan-B-cell antigen CD19 and for the CD3 subunit of the T cell receptor complex. Clinical efficacy of blinatumomab has been demonstrated in patients with therapy-refractory non-Hodgkin's lymphoma (NHL) and patients with B-precursor acute lymphocytic leukemia (ALL). Complete and partial responses have been observed in all 13 evaluable patients with indolent NHL treated with 0.06 mg/m² per day, and clearance of bone marrow from minimal residual disease in 16 out of 20 evaluable patients with ALL treated with 0.015 mg/m² per day.

Blinatumomab is a first generation BiTE, which cross-reacts only with respective antigens from chimpanzee. In order to facilitate *in vivo* safety testing of this class of molecules, surrogate BiTE antibodies were generated that are cross-reactive with murine antigens. Likewise, so called hybrid BiTE antibodies, which bind CD3 on murine T cells and the human target antigen, were generated as tools for efficacy testing in tumor-bearing mouse models. The pharmacological characterization of BiTE antibodies includes in-depth analysis of their effects on tumor as well as on T cells. Various models are available for *in vivo* efficacy testing. For instance, xenotransplanted mice are reconstituted with human effector T cells after establishment of solid tumors. Strategies for nonclinical assessment of BiTE antibodies with specificity for CD19, EpCAM and EGFR will be presented.

A second generation of BiTE antibodies is now available, which is fully human in sequence and cross-reacts with a wide variety of non-human primates including cynomolgus macaques.

For references, abstracts and poster presentations, please visit: www.micromet.de

Speaker Abstracts

S16: Issues and challenges in pre-clinical safety testing of stem cells therapies

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Stem cells provide novel and exciting alternative therapies which will likely fulfil unmet medical needs especially in the area of age-related degenerative diseases.

The purpose of the presentation will be to review the different stem cell therapeutic modalities, review the regulatory guidance regarding stem cell development, highlight some of the challenges, and provide some examples of stem therapies currently in development.

Briefly, stem therapies can be broadly divided in autologous (from the same patients) and allogenic (from healthy human) while the type of stem cells used either derived from adult human tissues (e.g. mesenchymal stem cells or induced pluripotent stem cells) or from embryonic material (human embryonic stem cells derived from inner mass of the blastocyst).

The regulatory guidance around stem cell development is mostly underlined in 3 major regulatory guideline documents; The Public Health Act, Section 351 – Biologics from the FDA, the Regulation on Advanced Therapy Medicinal Products (ATMP) EC No: 1394/2007 and EMEA/319294/2010 (summary report on the EMA workshop on stem cells based therapies) from the EMA.

In terms of safety, the sponsor needs to assure that the cells do not have the potential to cause teratoma, migrate outside their site of injection/implantation, and finally assess their immunogenic potential.

There are a few clinical trials currently underway using stem cell therapies. As scientists, industry, and regulators are starting to shape up this area, it is essential, early on, to start the dialog to share the knowledge around the success and pitfall of such an approach.



Speaker Abstracts

S17: Biologic Therapeutics: Evolution in U.S. and European Regulatory Approaches to Risk Assessment

Lauren E. Black, Ph.D.

Senior Scientific Advisor, Charles River

Undoubtedly, monoclonal antibodies (MoAbs) are a class of well-characterized biologics that have revolutionized clinical management of serious diseases. They are now broadly recognized as pragmatic, targeted, economical, and effective therapies. These drugs are based on natural human protein structures and, as expected, their morphologic pathology changes may be subtle. But their virtues (targeted receptor binding) can cause harm through exaggerated actions or through unknown mechanisms. The risk of biologic treatment does not lay in reactive metabolites (usually there are none). The risks lay in deploying a poorly reversible, selective antagonist into a complex pathway, where the drug can overwhelm balanced controls, or unintentionally knock out a housekeeping function. Because MoAbs can have half-lives of weeks or months, it is no wonder that the body cannot always adapt by using its relatively fragile counter-regulatory controls.

We have learned these harsh lessons from both U.S. and European human trials where we saw first saw elevated TB from Remicade and cytokine storm from TGN1412. We would all hope to predict these events during preclinical programs. But can we really expect to understand comparative physiology and pathophysiology well enough to know when to trust our animal models, and when we must work harder to construct a 360 degree view of drug risks? An ex-FDA pharmacologist shares her perspectives on this “translational step” and why no regulatory guidance document can assure good prospective toxicology program design or offer the secret key to study interpretation. This talk will appeal to pathologists’ original veterinary training and advocate for systems and weight-of-evidence approaches to risk assessment. The veterinary pathologist, when informed of the drug’s mechanism of action and exposure profile, can help integrate clinical observations, time and dose-linked changes, and clinical pathology to achieve a more complete picture of risks and thereby, contribute substantially to mitigation of human risks.

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Speaker Abstracts

S18: Roadmap of a VLP vaccine for Chikungunya virus

Srinivas S. Rao DVM, PhD, MBA, Diplomate ACVP

Since its reemergence in Kenya in 2004, Chikungunya virus (CHIKV) has infected millions of people in Africa, Europe and Asia. The severity of the disease and the spread of this alphavirus present a serious public health threat in the absence of vaccines or antiviral therapies. We have shown that selective expression of viral structural proteins produces virus-like particles (VLPs) *in vitro* that resemble replication-competent alphaviruses, but are incapable of causing infection. Immunization with these VLPs in rhesus macaques elicited high-titer neutralizing antibodies that protected against viremia after high-dose challenge. In addition, passive transfer of antibodies into immunodeficient mice protected against subsequent lethal CHIKV challenge, indicating a humoral mechanism of protection. While animal studies suggest VLPs are efficacious, further toxicologic studies are required to establish the safety of this vaccine. In the presentation we will discuss the experimental schema, methodology, results, safety and toxicologic implications of the CHIKV experiments in the context of current licensed VLP vaccine.



Speaker Abstracts

S19: Case Presentation: Rat Respiratory Virus

Charles B Clifford, DVM, PhD, DACVP

Charles River Research Animal Diagnostic Services

This “compiled” case will present typical findings of the most common cause of infectious pneumonia in rats. Both actual case material and experimental results will be presented. Since the diagnosis of this disease can currently be made only by histopathology, the diagnostic criteria will be discussed with some emphasis on differential diagnosis. Although the disease was first recognized in the mid-1990s, the etiologic agent is still unknown; rat respiratory virus is only a working name for an agent that may or may not actually be a virus, be limited to the respiratory system or infect only rats. Current knowledge regarding transmission and histologic progression of this disease will also be summarized.

Speaker Abstracts

S20: Therapeutic Vaccines, Adjuvants and Vaccine Delivery Systems

Michaela Sharpe

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Traditional prophylactic vaccines generate antibody-mediated immunity to protect against disease. Vaccines are now being developed in which the administration of the vaccine induces an immune response that eliminates or reduces an antigen or induces tolerance to it (e.g. autoantigen); the so called therapeutic vaccination. To be successful a therapeutic vaccine will need to overcome immune tolerance while controlling dysregulation and/or deleterious effects of immune activation. Unwanted T cell activation and undesirable off target effects need to be minimised. Therapeutic vaccination requires an acceptable balance between an immune response that controls/destroys a target cell or molecule and a pathological immune response that results in detrimental tissue damage. Safety considerations include evaluation of reduced antigen level effects, analysis of antibody-dependent-cell-mediated cytotoxicity, and reversibility of the induced immune response and/or unwanted T cell responses.

Increasingly delivery systems and adjuvants are being considered to improve both prophylactic and therapeutic vaccines. Alternative routes of administration from the traditional intramuscular injection, aim to deliver vaccines to highly immunological tissue and there by enhancing the induced immune response. Where as adjuvants are agents that accelerate, prolong or enhance an immune response to a co-administered vaccine. In both situations it is important to balance maximum immune stimulation with minimal adverse events. Effects can be seen locally at the injection site or systemically, such as cytokine induced stimulation of the immune system, pyrogenicity and autoimmune disorders. All have been seen experimentally and can prevent the use in humans

To develop successful therapeutic vaccines, delivery systems and adjuvants, two key hurdles must be overcome; safety and efficacy. Safety concerns cause the most difficulty in achieving efficacy.

Speaker Abstracts



The BSTP Chirukandath Gopinath Lecture

S21: Pathology of Injected Biopharmaceuticals: How well do animal studies predict clinical outcomes

Jeffery Engelhardt

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The value and predictive power of nonclinical studies for potential effects of investigational medicinal products in humans is often debated. The subject of general predictivity of animal toxicity studies has been addressed on several occasions. The results of these investigations indicate that dermatologic reactions in humans are often poorly predicted by animal studies. An analysis of the literature and my personal experience with respect to the type of biologic, histopathological evaluation in animal studies, and observations in clinical studies was conducted for a variety of parenteral biotherapeutic agents. The outcome of this analysis indicated that biologics tended to behave as polymeric materials or foreign bodies at the injection site in animals and likely in man. The residence time of the protein at the injection site was the greatest determinant for the severity of the injection site reaction in animals followed by physical form and volume. There was, however, a large amount of variability in the histological presentation among the different biotherapeutics. Human pathology at the injection site was also quite variable and rarely correlated with the lesions present in the animal species. This lack of concordance indicates that the occurrence of haemorrhage, oedema, and/or inflammatory cell infiltration with or without fibroplasias/fibrosis in the animal studies should be considered as only a signal for potential effects at the injection site in the clinical programme. The presence of injection site reactions in animal studies is not of sufficient sensitivity to be used as a reason to discontinue development of a candidate biotherapeutic. Findings should only be used to help guide the clinical development.

Speaker Abstracts

S22: Cytokine Release by Therapeutic Monoclonal Antibodies - Defining the Mechanism and Minimizing the Risk in FIH studies

Frank R. Brennan PhD

Novartis Biologics, Translational Sciences and Safety

Therapeutic monoclonal antibodies (mAbs) have the potential to trigger systemic Cytokine Release Syndrome (CRS), usually after the first infusion, either by cross-linking and clustering of the target on immune cells by the Fab arms resulting in effector (T, B, NK) cell activation, by interaction of the Fc region with Fc gamma receptors (Fc γ R) on NK cells and neutrophils, or a combination of the two. Though multiple cytokines may be present, the classic signature of CRS consists of the pro-inflammatory cytokines TNF α , IFN γ and IL-6. The systemic and local presence of these molecules and the associated inflammation and haemodynamic effects damage tissues and organs, and can result in disseminated intravascular coagulation, organ failure and death if left untreated. Analysis of serum cytokines in humans provides a primary means of monitoring the development and resolution of this syndrome, with supportive evidence provided by clinical signs/symptoms, peripheral blood differential cell counts, and flow cytometric analyses. However the most severe clinical sequelae of CRS do not occur in animal models, in spite of the generation of high systemic levels of cytokines in some cases. Thus in vitro studies with human cells may be of more value in trying to assess the risk of CRS prior to First-in-Human studies. This presentation will describe the use of in vitro cytokine release assays (CRAs) to identify the potential of an immune cell targeting mAb to induce cytokine release in humans and to define the mechanism of this cytokine release. The incorporation of the CRA data into the Minimum Anticipated Biological Effect Level (MABEL) calculation and in the selection of a human starting dose, as well as clinical risk mitigation strategies, are discussed.

Speaker Abstracts

S23: Biodistribution of Biopharmaceuticals & Cross Reactivity Studies

Jennifer L. Rojko, DVM, PhD

DACVP and Shari A. Price-Schiavi, PhD, DABP, Charles River Laboratories, USA

Biodistribution studies are used to localize test articles within tissues and/or organs. Standard methods for biodistribution studies do not provide information about actual cellular and/or subcellular localization of a given test article. Immunohistochemical (IHC) methods can be used to demonstrate cellular and subcellular localization of many monoclonal antibody (mAb) test articles as well as other protein/peptide, nucleotide-based, or small molecule drugs when an appropriate detection reagent is available. IHC-based studies also are used to determine whether or not a given test article is present at the site of a lesion. Additional IHC staining can be performed to determine if the lesion involves an immune or inflammatory response (e.g., formation/deposition of immune complexes).

When monoclonal antibodies (mAbs) are administered parenterally, the mAb becomes incorporated into the endogenous IgG pool and is distributed, transported, taken up by cells, cleared and catabolized similarly to endogenous IgG. Factors include effect of cardiac output, concentration-gradient, type of endothelium, accumulation in interstitium, FcR and non-FcR-mediated uptake and clearance, barriers to transport, and catabolism. Additionally, the mAb distribution can be altered by mAb CDR-mediated binding, development of anti-mAb antibodies and/or formation of mAb-containing immune complexes, or by structural alterations in the mAb molecule (e.g., conjugation with toxin). Examples will be presented of these different types of mAb biodistribution patterns. Other examples will compare in vitro tissue cross-reactivity (TCR) staining patterns with in vivo biodistribution staining patterns.

Slide Seminar Abstracts

Case Presentations

Dear Colleagues,

Yes, we know you are used to find the details to the case presentations here – but this year it is different: Since we have this interactive voting system, we decided to keep the results of the cases as a secret, and that you then will receive the results and all necessary information to the respective cases after the congress as a pdf to complete your abstract book at home. We kindly ask for your understanding.

Please use the space below to make your own notes!

Poster Abstracts

Po1: Nasal and pulmonal translocation of fine and nano TiO₂ particles after nose-only inhalation in rats

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Introduction: Particulate matter is of great concern because of its effects on public health. Fine particles and nanoparticles are widely distributed throughout the environment. They are used in a variety of industries and subject of regulation for workplace safety. Nanoparticles are supposed to cause more severe biological alterations compared to their fine counterparts due to their high surface-size-ratio. Those effects may also play a role in the pathogenesis of neurodegenerative diseases such as Alzheimer or Parkinson disease.

Titanium dioxide (TiO₂) is an insoluble and nearly inert material. Its fine particles cause only minimal changes. In contrast, TiO₂ nanoparticles can exhibit more severe inflammatory reactions in the lung, predominantly of the acute type. The aim of the present study was to investigate the differences in translocation and morphological alterations in the nasal cavity and lung after inhalation of fine and nano TiO₂ particles by light and transmission electron microscopy.

Materials and methods: Female Wistar rats were exposed to 10 mg/m³ nano TiO₂, 45 mg/m³ fine TiO₂ and clean air, respectively. The inhalation was performed 6 hours per day for 21 consecutive days resulting in predicted lung loads of 1.3 and 5.8 mg/lung. Animals were euthanized after a recovery phase of 3, 28 or 90 days.

Results: Using light microscopy, brains revealed no morphological alterations and no particles were detectable. Within the nasal cavities fine particles and nanoparticles were found in small amounts in macrophages and epithelial cells without any inflammatory reaction. In the lung, moderate amounts of particle-laden macrophages, minimal interstitial mononuclear cell infiltration occasionally accompanied by some granulocytes, and very slight bronchiolo-alveolar hyperplasia were found. Transmission electron microscopy of the lung revealed cytoplasmic deposition of fine and nano particles exclusively in alveolar macrophages and to a lesser extent in type I pneumocytes.

Conclusion: In contrast to previously published data, differences in translocation behavior or morphological alterations between nanoparticles and fine particles have not been detected in the present study so far. This might be due to the relatively low aerosol concentrations of TiO₂ resulting in only a slight overload situation within the lung and the inert character of the dusts.

Poster Abstracts

P02: Sampling the primate larynx in inhalation toxicity studies

Charlotte Keenan¹, Rodney Miller³, John Bowles¹, Deon Hildebrand¹, Jan Klapwijk², David Lewis², Beverly Maleeff¹, Tom McKeivitt¹, Heath Thomas¹, Jerry Hardisty³, David Sabio³

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Lesions observed in the larynges of rats and mice that are induced by inhalation of xenobiotics occur with some frequency and have a sensitivity related to anatomic site specificity. Trimming protocols have allowed for the precise trimming and tissue preparation of rodent larynges such that the transition zone of the posterior epiglottal squamous epithelium with the laryngeal respiratory epithelium has been determined to be the most sensitive anatomic location for lesion development. This anatomic specificity of lesion induction in rodents has generated interest in developing a protocol that allows for the precise and consistent collection, trimming, and histologic preparation of uniform sections of the larynges of the primate. The consistency in trimming larynges allows for a better understanding of the epithelial types that are expected to be seen at the various levels, clearer identification of the zone of transition of squamous epithelium to respiratory and an increased likelihood of determining the areas of increased sensitivity of the epithelial types.

Poster Abstracts

P03: Summary of laryngeal lesions in Fischer Rats and B6C3F1 Mice in chronic inhalation studies

Rodney A. Miller, EPL; Gabrielle A. Willson, EPL; Glen E. Marrs, EPL; Ronald A. Herbert, NTP; and Jerry F. Hardisty, EPL

A comprehensive review summarizing data of laryngeal lesions recorded in a large series of studies would be of value as a reference for future microscopic evaluations of rat and mouse larynges.

Microscopic findings in the larynges taken from forty-eight finalized reports of peer reviewed chronic (two year) inhalation studies were summarized.

Incidences of non neoplastic and neoplastic laryngeal lesions recorded in control and treated, male and female, rats and mice are detailed. Spontaneous and induced lesions include squamous metaplasia, inflammation and hyperplasia. The base of the epiglottis was the most common site affected and thus considered to be the most sensitive site. Induced lesions were observed anterior and posterior to the base of the epiglottis but never to the exclusion of the base of the epiglottis.

One of the more commonly diagnosed test material induced lesions of the larynx was squamous metaplasia. Frequently, when chemical-induced laryngeal squamous metaplasia is observed, the possibility of progression to neoplasia is of concern. In the review of this large series of studies, there was no indication of progression of test material induced squamous metaplasia to laryngeal neoplasia.

Poster Abstracts

Po4: Design-based stereology in lung toxicology

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Stereological methodology is critical to effective sampling in lung tissue. This poster provides an introduction to the basic principles of design-based stereology with emphasis on its practical application to the lung. We discuss experimental design, specimen preparation, sampling techniques, use of stereological probes, and estimation of stereological parameters. Examples of applications to animal models of lung disease will be given. The principles and recommendations follow the recently established “standards for quantitative assessment of lung structure”, developed by a task force of the American Thoracic Society and European Respiratory Society, which have been published as an official ATS/ERS research policy statement (Hsia CCW, Hyde DM, Ochs M, Weibel ER. *Am J Respir Crit Care Med* 2010;181:394-418). Sampling approaches and best practices in software will be described.



Poster Abstracts

P05: Basic and advanced image analysis assessment of the lung

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The measurement of open space structures in tissue sections (e.g. fat vacuoles, alveoli, vessels) is a vital aspect of histopathological assessment. Lung anatomic structures such as alveoli and bronchiolar measurements are important in a number of research and disease areas, but it is often a time and resource-consuming task to obtain quantitative measurements of these structures. With the power of digital pathology and the emerging technology of automated quantitative image analysis algorithms, the researcher can now rapidly obtain reproducible, non-biased quantitative information. Basic and advanced image analysis tools will be compared and contrasted in the assessment of the measuring of clear spaces such as alveoli and bronchiolar regions.

Poster Abstracts

Po6: Respiratory tract responses in Wistar and BN rats, sensitized and challenged by inhalation with the contact allergen dinitrochlorobenzene (DNCB)

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All chemical respiratory allergens studied so far can also induce skin sensitization/allergy in test animals. The question is if in turn contact (skin) allergens can induce allergy in the respiratory tract. The contact allergen dinitrochlorobenzene (DNCB) was tested first in Th2-prone Brown Norway (BN) rats, using a protocol that successfully identified chemical respiratory allergens like trimellitic anhydride. Dermal sensitization induced DNCB-specific IgG in serum. A subsequent single inhalation challenge with DNCB did not provoke apnoeic breathing or allergic inflammation in the respiratory tissues (signs of respiratory allergy), but the allergy-associated genes for Ccl2 (MCP-1), Ccl4 (MIP-1beta), Ccl7 and Ccl17 were upregulated in lung tissue. Next, DNCB was tested in Th1-prone Wistar rats. Again, a single inhalation challenge in sensitized rats did not provoke apnoeic breathing, but induced a minimal lymphocytic infiltrate in the nasal tissues and larynx. Repeated inhalation challenges (twice a week for 4 weeks) in Wistar rats induced DNCB-specific IgG antibodies in serum and a pronounced, predominantly lymphocytic, inflammation in the nasal tissues and larynx. The inflammation may be the upper respiratory tract analogue of hypersensitivity pneumonitis/allergic alveolitis. The relevance of these findings to man, and possible progression of the airway inflammation should be investigated to support or dismiss discrimination between contact and respiratory allergens in relation to respiratory allergy.

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Poster Abstracts

P07: Lungworm-associated changes resembling *Filaroides hirshi* infection in Beagle dogs during the past 15 years

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The metastrongyloid nematode infection with *Filaroides hirshi* in dogs was known since 1973, first described by Hirth for a Beagle colony in the US. In the 80's, lung lesions characterized by granulomatous inflammation, indicating parasitic origin, were frequently observed although anti-parasitic treatment was routinely performed. In the 90's, a cohort from one company in Germany reached an approx. 96% incidence of macroscopic and microscopic lesions ranging from granulomatous inflammation to tumor-like lesions. Meanwhile, in several European countries and in the US, *Filaroides hirshi* infections were detected by several investigators. Consequently, an appropriate treatment (ivermectin) was developed.

The incidences of lungworm-associated pulmonary changes decreased rapidly after treatment. Anecdotal observations of recent studies indicated a recurrence of pulmonary changes in beagle dogs on study. Therefore, retrospective data review was performed at the Toxicology Department of Abbott GmbH & Co. KG, Ludwigshafen, Germany, to elucidate the occurrence of lungworm-associated lesions between 1996 and 2010. For this purpose, the macroscopic and microscopic data tables of all dog studies using dogs of US and European breeders were integrated in this evaluation. The majority of lesions was observed on gross pathology. Whereas in subtle cases pleural, subpleural or parenchymatous focal or multifocal discolorations were noted, the more extensive lesions appeared as firm nodules in the lungs. On histopathology, typical lungworm-associated changes consisted of bronchiolo-alveolar hyperplasia, fibrosis, inflammation and cellular infiltrations. Compared to the situation before ivermectin treatment against *Filaroides hirshi* the incidences and the extent of lung lesions have decreased in magnitude. However, since the lesions occurred again from time to time, clear descriptions as such are considered critical for toxicological assessments in drug development.

Poster Abstracts

Po8: Sampling of the Cynomolgus monkey nervous system in general toxicity studies

Ingrid D. R. Pardo¹, Daniel Morton¹, Robert Garman², Jerry Hardisty³, Rebecca Moore³, David Sabio³, Klaus Weber⁴, Walter F Bobrowski³, Cathy Tabor¹, Natalka Kopczyk¹, Denise Pernal¹, Laura Kearney¹, Kim Kowsz¹

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For general toxicity studies, a technique was designed to consistently sample the most important neuroanatomic regions of the brain, spinal cord and peripheral nerve of cynomolgus monkeys using a limited number of blocks and slides. Using the pons as a landmark brain, the entire fixed brain was cut dorsoventrally into multiple cross sectional slabs 4 mm in thickness. For microscopic evaluation, six sections of the brain at the levels of the frontal pole, anterior commissure, anterior thalamus, posterior thalamus, middle of the cerebellum with brainstem and occipital lobe were trimmed to fit in standard tissue cassettes. Cross and oblique sections of the spinal cord including the dorsal root ganglion and dorsal and ventral nerve roots were obtained at the level of C1-C4 and T12-L4. Cross and longitudinal sections of the sciatic nerve were also obtained. This technique offers a consistent and reliable method to routinely sample most of the important regions of the central and peripheral nervous system of monkeys using 9 blocks. This method is readily adaptable to other species of nonhuman primates, dogs, and minipigs and can be performed by histotechnicians with minimal neuroanatomical knowledge.

Poster Abstracts

P09: An extended trimming procedure of the minipig brain

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In pre-clinical toxicology studies, the standard examination of the minipig brain includes examination of 6 brain samples, where the cerebrum, hypothalamus, midbrain, cerebellum, pons and medulla can be examined.

Especially in the testing of CNS-active compounds, it is considered useful to be able to microscopically examine more structures or the same structures on different levels. This can be achieved using an extended trimming procedure.

We have many years of experience using the minipig as the non-rodent species in toxicity testing of potential novel pharmaceuticals, and have now implemented this extended trimming procedure of the brain of the minipig.

The extended trimming procedure makes it possible to obtain a total of 16-17 samples of the minipig brain, where the first section is a cut through the olfactory bulb, followed by coronal slices of approximately 3 mm. The desired slices are then selected by visual evaluation. When using the extended trimming procedure, the olfactory region, the hippocampus, limbic cortex, piriform cortex and the amygdala can be examined in more detail. It is also possible to examine the deep cerebellar nuclei, however these can be slightly more complicated to localise in the slices obtained from the minipig. The standard stain is Haematoxylin & Eosin; however Fluorojade B can also be used for more specific histological staining of neurons undergoing degeneration.

At LAB Research the new extended trimming procedure of the minipig brain has now been implemented as a standard procedure.

Poster Abstracts

P10: Alternative dog skin sampling for routine toxicology studies

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Currently, the industry standard dog skin sample for routine general toxicology studies is taken in the inguinal region together with the mammary gland.

The dog abdominal area is usually sparsely haired and subject to trauma by friction. Histologically, the hair follicles density is usually low (with frequently abnormal morphology), the hypodermis can be totally absent (replaced in female by mammary gland). Thus this sample is not considered to represent normal/typical skin.

In order to propose an alternative sampling area, a variety of sites (midline ventral thorax, flank, neck, midline dorsal thorax, midline dorsal abdomen) were selected and examined for 1 male and 1 female control dogs from a standard 28-day study.

One longitudinal and one transversal section were prepared for each site and stained with H&E and PAS.

The following endpoints were evaluated:

- Hypodermis development
- Apopilosebaceous units density and morphology
- Hair cycle

When compared to the inguinal skin, all the evaluated samples showed better microscopic characteristics. The dorsal samples having the best characteristics (hair density, muscle).

Though morphologically acceptable, the flank and ventral samples were not selected. These areas were considered to be prone to friction and subject to scratching.

The midline abdominal dorsal skin was selected as the easiest to sample at necropsy without impacting the current procedure.

This work allowed us to select a skin sample (midline dorsal abdomen) considered to better represent the normal morphology than the inguinal skin.

We added this sample to our in-house studies, in addition to the inguinal one (for the mammary gland).

Preliminary results showed a significant decrease in the inter-individual variation (sample more consistent). We will continue to monitor the results to validate the improvement.

Poster Abstracts

P11: Spontaneous lesions of the skeletal muscle in the Göttingen minipig

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Since the Göttingen minipig was introduced more than 20 years ago, the use of this species in biomedical research has increased tremendously. The Göttingen minipig is bred in barrier facilities and microbiologically defined due to a well established and extensive health monitoring program. As the Göttingen minipig is microbiologically defined, breeding is aimed at keeping the minipig as outbred as possible and because the pig is close to man with respect to many anatomical and physiological features, the Göttingen minipig is thus well suited for use in pre-clinical toxicity testing of potential novel pharmaceuticals.

However, even though the Göttingen minipig is used extensively in the pre-clinical testing of new pharmaceuticals, the literature on common background pathology is still scarce in this species, limiting the documentation in the evaluation of minipig studies to experience of the individual pathologist and access to historical data.

Based on the Lab Research historical database 2000-2009, we present the incidence of commonly described incidental lesions in the skeletal muscle of Göttingen minipigs.

Poster Abstracts

P12: Intramuscular injection sites: is the quality of the diagnosis improved when more than one tissue section is examined?

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The standard procedure for microscopic evaluation of intramuscular injection sites at our Laboratory involves the examination of three tissue pieces from each site, in order to maximise the chance of examining the area where the injected material was deposited. This is laborious, however, so we retrospectively studied the extent to which the quality of the diagnosis is maintained if only one section per injection site is examined.

Two rabbit and two rat studies with intramuscular injection of the vehicle or test item were reviewed by one pathologist. In rabbit studies (160 animals and 800 injection sites) intramuscular injections were given in the dorsolumbar, quadriceps femoris or gluteus medius muscles and each site received one injection. In rat studies (120 animals and 320 injection sites) injections were given under anaesthesia in the quadriceps femoris or gluteus medius muscles and each site was injected 4 or 7 times. For both species, the zone was previously clipped and the injection was performed in the centre of this area, which was sampled at necropsy. Three pieces were trimmed from the sample, each piece separated by 0.3 cm in rats and 0.5 cm in rabbits. Piece 1 was in the middle, piece 2 lateral to it and piece 3 medial to it. Sections from each piece were re-examined and each of the original diagnoses, which summarised findings in all 3 sections together, was assigned to the section(s) where it was observed. The studies in each species were pooled, but control and treated animals were evaluated separately. Based on the similar nature and number of findings between sexes, males and females were pooled together. For each animal, the percentage of findings found on each section when compared to the original diagnosis was calculated.

Section 1 had a statistically significantly higher number of relevant findings when compared to sections 2 and 3 in treated rats and rabbits. In treated rats more than 85% of the original diagnostic features were found on this section. In rabbits, this percentage reached more than 75%. In control rats and rabbits, section 1 had a higher or equal number of findings than the other sections.

This retrospective study reveals that examination of more than one tissue piece does not greatly improve the quality of the diagnosis. One middle piece is considered to be sufficient to provide an accurate representation of lesions at intramuscular injection sites.

Poster Abstracts

P13: A case of severe subepicardial inflammation as part of the entity “dystrophic cardiac calcinosis” in a young BALB/c Mouse

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Spontaneous mineralization of the heart known as “dystrophic cardiac calcinosis” is a common finding in laboratory mice.

The incidence, location and severity is influenced by multiple factors such as strain, age, sex, and diet. Especially inbred DBA/2, C3H, and BALB/c are frequently mentioned as strains commonly affected by this condition.

Without clinical abnormalities during life time, cardiac mineralization is a typical incidental finding at necropsy. The presented case was part of 110 BALB/c control mice (breeder: Charles River Deutschland, Sulzfeld, Germany) maintained under the standardized environment of a laboratory animal house. None of the mice revealed any clinical signs or diseases during their life time. The animals were killed at the age of six, eight, ten or eleven weeks. At necropsy gross lesions of the heart were detected in 15 to 25% of the male and female BALB/c mice. These macroscopical findings ranged from single pale foci to widespread white plaques which were predominantly seen in the right ventricular wall. Histological changes ranged from single myocyte degeneration, minimal mononuclear cell infiltration, minimal fibrosis, and minimal mineralization to marked plaque-like mineralization and fibrosis. All alterations were located epicardially or were found in the adjacent myocardium. Furthermore, using light microscopy, one six weeks old male animal exhibited a marked cardiac inflammation consisting predominantly of macrophages. The inflammatory process affected the subepicardial area of both ventricular walls. These lesions were interpreted as severe acute alterations belonging to the entity “dystrophic cardiac calcinosis”. The lesions in the heart of this mouse in comparison to the findings of the other animals of the BALB/c mice group will be presented.

Poster Abstracts

P14: Spontaneous retinal degeneration in Hsd:ICR(CD-1) Mice

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The mouse is one of the most widely used animal species in the nonclinical safety and toxicological studies. Knowledge of the background lesions in different mouse strains/stocks is very crucial, and on the basis of this information, the identification and differentiation of the induced alterations from the spontaneous ones are also essential.

We observed a retinal lesion with more than 40% incidence in a 14-day oral toxicity study in mice. On the histological examination, retinal degeneration (loss of rod and cone cells, absence of the photoreceptor layer, outer nuclear layer and outer plexiform layer) was detected as a spontaneous finding in 15/25 (60.0%) males and in 12/25 (48.0%) females. The mice were from the Hsd:ICR(CD-1) stocks, the breeder was HARLAN Italy. The age of the animals at the beginning of the study was approximately 6 weeks.

We found one reference in the scientific literature concerning this stock and this lesion: in that publication the incidence of the retinal degeneration was 17/30 (56.7%) in males and 9/30 (30.0%) in females, and the authors mentioned the incidence was nearly twice higher in males than in females. Based on our data, the difference between sexes was somewhat less. Comparing our results with the literature, the incidence in males was nearly the same (60.0% vs. 56.7%), while among the females the lesions were more frequent in our study (48.0% vs. 30.0%).

Our results emphasize that this stock, the Hsd:ICR(CD-1) should be used with caution in toxicological studies with knowledge of these incidences. In addition, pretest ophthalmologic screening for this pre-existing condition prior to the start of a study is highly recommended by the literature.



Poster Abstracts

P15: Pathology of ocular irritation in the bovine corneal opacity and permeability (BCOP) assay

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The purpose of this study was to assess the extent to which this fundamental relationship exists for bleaching agents in Bovine Corneal Opacity and Permeability (BCOP) assay. This evaluation was used to compare depth of injury with the opacity and permeability scores and to identify lesions that were not fully expressed in opacity or permeability changes. The degree of epithelial damage is reflected in the increase in fluorescein permeability value. Histological evaluation was performed on the three layers of the cornea to provide a direct measure of the depth of injury. Also, the opacity and permeability endpoints (generally combined into an “in vitro score”) have been able to identify the epithelial and stromal changes associated with these types of damage. Membrane lysis, protein coagulation, and saponification are common modes of action that lead to ocular irritation. Despite differences in the processes leading to tissue damage, has been shown to depend on the extent of initial injury. This evaluation was used to compare depth of injury with the opacity and permeability scores and to identify lesions that were not fully expressed in opacity or permeability changes.

Poster Abstracts

P16: Immunohistochemical Characterization of Kidney Lesions in Cynomolgus Monkeys using a Triple Staining Method

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A new test compound was given by oral gavage for up to one month to cynomolgus monkeys. The kidney was identified as one of the main target organs. After being dosed with high doses of the test compound for 4 to 7 days, multifocal degeneration of kidney tubuli with necrosis of tubular epithelial cells was present using H&E staining. Affected tubuli were markedly dilated and filled with neutrophils and debris. Chronic administration of lower doses led to focal tubular degeneration and multifocal regeneration of tubuli associated with a mild interstitial inflammation. While the tubuli showed severe changes, glomeruli remained unaffected.

In order to better characterize the lesions and identify a potential biomarker, the aim was to localize the lesions to particular nephron segments and correlate histopathological changes with clinical chemistry results. A triple stain immunohistochemistry method was developed, using antibodies against aquaporin 1, aquaporin 2 and calbindin D28K. Proximal tubules and collecting ducts stained positive for aquaporin 1 or aquaporin 2 respectively. Calbindin D28K labeled distal tubules and collecting ducts in the cortex, medulla and papilla. This is in contrast to other species where calbindin D28K is predominantly expressed in the distal tubules with weaker expression in cortical collecting ducts. Using this triple staining method, the tubular lesions could be localized to the distal tubules and collecting ducts. This allowed us to use additional molecular techniques to identify a potential biomarker with utility in the clinic.



Poster Abstracts

P17: Renal dysplasia in Beagle dogs: Four cases

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Anomalies of renal development comprise abnormalities in the amount of renal tissue (agenesis and hypoplasia), anomalies of renal position, form, orientation and renal dysplasia. There are previous reports of canine renal dysplasia in different breeds, however none in the Beagle breed: this is the first report of renal dysplasia in this breed of dog. Morphologic descriptions of the range of microscopic features observed in four cases of renal dysplasia from preclinical studies in laboratory Beagle dogs are presented (including persistent primitive mesenchyme, persistence of metanephric ducts, asynchronous differentiation of nephrons, atypical tubular epithelium), along with a basis for the classification of the lesion.

Poster Abstracts

P18: Thyroglobulin expression in a poorly differentiated thyroid follicular carcinoma in the CD-1 mouse

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Spontaneous thyroid gland tumours are relatively uncommon in most mouse strains. Here we describe a poorly differentiated thyroid tumour in a control CD-1 mouse from a carcinogenicity study. At necropsy, a large unilateral mass was recorded in the thyroid gland. On histological examination, a thyroid neoplasm was identified together with cellular infiltrates in the lung that were considered to be metastatic deposits. The primary thyroid tumour displayed two disparate morphological areas. The majority of the tumour was semi-lobulated and comprised large round to oval eosinophilic cells arranged in packets and occasionally forming acinar structures with eosinophilic contents. A second area contained elongated cells with indistinct cell boundaries arranged in sheets and frequently displaying a whorling pattern. The pulmonary deposits were made up of solid nests of large eosinophilic cells.

In the primary mass, consistently strong immunohistochemical staining for thyroglobulin was observed in the cytoplasm of eosinophilic and acinar cells. In contrast, few thyroglobulin signals were noted in areas of elongated cells. Strong to moderate thyroglobulin staining was also seen in all pulmonary deposits. No positive staining for calcitonin was present in neoplastic cells in either the primary thyroid mass or secondary deposits. The morphological appearance of the tumour and results of immunohistochemical staining are consistent with a diagnosis of follicular cell carcinoma. This is possibly the first report of thyroglobulin expression being used to confirm the histogenesis of a thyroid neoplasm in the mouse. We propose that immunohistochemistry for thyroglobulin and for calcitonin markers is a useful tool in the diagnosis of such tumours.

Poster Abstracts

P19: VEGF inhibitors toxicity in preclinical safety studies – an overview

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The discovery of the critical role of angiogenesis in tumor progression and metastasis, alongside the unraveling of many important molecules controlling new vessel formation, prompted many pharmaceutical and biotechnology companies to develop novel treatment strategies aimed at inhibiting these critical processes. A pivotal role for cell surface tyrosine kinase receptors (RTKs) for vascular endothelial growth factors (VEGFs), fibroblast growth factors (FGFs) and platelet derived growth factors (PDGFs) in angiogenesis and neo-vascularization associated with solid tumors was demonstrated, and has been thoroughly investigated for therapeutic targeting in the past two decades.

Although blood vessels in the healthy adult are considered stable and quiescent in most tissues, the disruption of specific VEGF signaling by monoclonal antibodies has been shown to result in toxicity in specific organs relying on angiogenesis for maintenance and development. In particular, changes in the renal glomeruli with associated proteinuria, hypertension, a reduction of number of corpora lutea in the ovary and physal growth plate cartilage dysplasia in long bones have been associated with a specific VEGF-mediated pharmacological target organ effect, having been observed in nonhuman primates or patients treated with this class of compounds. The development of small molecule Receptor Tyrosine Kinase inhibitors, characterized by a lower selectivity (multi-targeted RTK inhibitors) resulted in a widened spectrum of compound-induced lesions in a number of organ systems. Additional findings reported to occur in different animal species (rats, monkeys and dogs) include periosteal cartilage and bone formation in the femur and sternbrae, hemorrhage and necrosis in the adrenals, increase in liver enzymes, dentin degeneration in the teeth, acinar degranulation in exocrine glands and atrophy of the squamous epithelia (tongue, esophagus and vagina). This poster provides a few examples of the effects of some antiangiogenic multi-targeted RTK inhibitors in preclinical studies and their correlation with what has been described in patients.

Poster Abstracts

P20: Pathology and Quality Risk Management: A promising partnership

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Quality Risk Management (QRM) can be a valuable component of an effective quality system in pharmaceutical industry, including Toxicologic Pathology. Regulators are creating the environment for industry (ICH Q9, June 2006), emphasizing the ability of risk management to facilitate (but not obviate!) industry's obligation to comply with regulatory requirements.

QRM Processes were used in Johnson & Johnson's Preclinical Tox/Path/LAM Department in order to optimize the Histology processes and the GLPQA audits, and to provide a framework for process improvements. The histology processes that were evaluated included the processing of tissues by histo-technicians and histological evaluation by the pathologists. Certain histo-technical processes, e.g. the handling of the data / materials generated by external business partners (CRO's) had grown historically towards less transparency and to rather complex document flows and were sometimes creating a bottleneck at the time of archiving.

To get a clear overview and a deep insight in all currently existing histology procedures (internal and external), very detailed deployment flowcharts were made by QA auditors, in close consultation with pathologists and histo-technicians. These practical deployment charts were critically compared to the written standard operating procedures (SOP's).

The following systematic strategy was then designed to improve (science-based) decision-making by gaining insight in risks (probability, impact) using QRM tools.

1. Determination of the critical steps in the different processes
2. Risk analysis on these critical steps with determination of the Risk Priority Factor; taking into account the impact, probability and detection rate
3. Brainstorm and define advises for process improvements with a better focus on priorities and resources

We experienced these tools as a great help for efficient teamwork, parallel thinking, stimulation of innovative ideas and thorough exploration of issues and opportunities.

As a tangible result, 7 SOP's, 13 Forms and 30 logbooks concerning histo-technical processing were reduced to 2 SOP's, 5 Forms and 11 logbooks, respectively. A better focused and consistent GLPQA audit approach for Histology raw data and reports was established, that has lead to more consistency within GLPQA.

This customized implementation of QRM and process excellence tools, embraced by both the histopathology department, QA and our external business partners, helped us to move, through innovative quality solutions, to a more strategic approach that has long-term impact and is proving that science, compliance and feasibility can go hand in hand.



Poster Abstracts

P21: Validation of whole slide image analysis algorithms for use in GLP studies

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The pharmaceutical industry is rapidly moving to usage of whole slide images in many areas of toxicologic pathology investigation. Most applications can either be classified as telepathology or quantitative analysis. In quantitative analysis, both stereology and whole slide analysis are critical emerging tools in providing a more quantitative basis to pathology assessment. This poster will address an approach developed for image analysis that will satisfy GLP requirements. These tools have seen widespread usage in non-GLP settings, including biomarker discovery, target validation, and predictive toxicology. There are many situations in GLP settings where a pathologist could utilize quantitative analysis if it was properly validated. We describe an approach for validation of image analysis, that balances reproducibility and documentation needs with real-life time constraints. This approach is divided into 5 areas: 1) Installation and Operational Qualification (IQ/OQ) of the Scanner or hardware acquisition device, 2) IQ/OQ of basic region of interest drawing tools, 3) IQ/OQ of automated region of interest tools using histology pattern recognition, 4) IQ/OQ of cell/area/object quantitative analysis, and 5) Performance Qualification of the entire analysis process.

Poster Abstracts

P22: Acrylamide-induced spinal cord axonopathy (The neuropathologic effects of different doses of acrylamide monomer on thoracic portion of the spinal cord of rats)

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Distal swelling and eventual degeneration of axon in the CNS and PNS have been considered to be the characteristic neuropathological features of acrylamide (ACR) neuropathy.

This study was conducted to determine the neurotoxic effects of different doses of ACR on the spinal thoracic axons of rat. To evaluate these effects in thoracic axons, *amino-cupric silver staining technique of de Olmos* and *electron microscopic* examination were performed to define the histopathological (argyrophilia) characteristic of axonal damage following ACR exposure at the T1 – T5 region of the rat spinal cord.

60 adult male rats (Wistar, approximately 250 g) were selected. Rats were housed in polycarbonate boxes (two per box) and randomly assigned to 6 groups (10 rats per exposure group). A total of 5 exposure groups (A,B,C,D,E) were exposed to 0.5, 5, 50, 100 and 500 mg/kg per day, respectively, for 11 days, i.p.. The remaining 10 rats were housed as control group (group F). Control rats received daily i.p. injections of 0.9% saline (3ml/kg).

As evidence of developing neurotoxicity, weight gain, gait scores and landing hindlimb foot splay (LHF) were determined. Weight gains were measured daily prior to injection. Gait scoring involved observation of spontaneous open field locomotion, and included evaluations of ataxia, hopping, earing and hind foot placement. Hind limb foot splay were determined 3-4 times per week. Gait score was assigned from 1-4.

After 11 days, two rats were randomly selected for silver staining, and two rats randomly selected for EM examination. The rats were dissected and proper samples from thoracic spinal cord were collected.

Results did not show any abnormal neurological behavior in groups A, B and F, whereas severe neurotoxicity was observed in groups C and D. Rats in group E died within 1-2 hours due to severe toxemia.

In histopathological studies based on *de Olmos* technique no argyrophilic neurons or processes were observed in stained sections obtained from thoracic spinal cord of rats belonging to groups A, B and F, while moderate to severe argyrophilic changes were observed in sections obtained from thoracic spinal cord of rats belonging to group C and D.

In *ultrastructural studies* some variations in the myelin sheath of injured axons including decompactation, interlaminar space formation, disruption of the laminar sheet, accumulation of neurofilaments, vacuolation and clumping inside the axolemma, and finally complete disappearance of laminar sheath were observed.

Poster Abstracts

P23: Molecular pathological mechanism in thioacetamide – induced hepatotoxicity

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It has been reported that treatment with thioacetamide (TAA) is inducing hepatitis in an acute phase and liver cirrhosis in a chronic phase in animal model, which is associated with oxidative stress and activation of hepatic stellate cells. In a previous study, we compared the gene expression profiles from TAA exposure and assessed the comparability between *in vivo* and *in vitro* test systems to elucidate its molecular pathological mechanism. By investigating global gene expression from both a mouse liver and a mouse hepatic cell line treated with TAA, we identified several common genes. Then we selected genes to validate them as potential biomarkers for hepatotoxicity based on their relevance in *in vitro* and *in vivo* systems. Three up-regulated, aquaporin 8 (Aqp8), glutathione peroxidase 1 (Gpx1), succinate-CoA ligase, GDP-forming, alpha subunit (Succg1) and two down-regulated, Dnaj (Hsp40) homologue subfamily C member 5 (Dnajc5) and tumor protein D52 (Tpd52) genes were tested for their effects *in vitro*. For characterization of gene function, short interfering RNA (siRNA) for each gene was synthesized and transfected into the mouse hepatic cell line BNL CL.2. Cell viability, mRNA expression level and morphological alterations were investigated. We confirmed that siRNA transfection induced down-regulation of mRNA expression of five selected genes. In general, siRNA transfection decreased cell viability at different degrees and induced morphological changes such as membrane thickening and alterations of intracellular structures. This suggests that these genes could be associated with TAA-induced toxicity. Furthermore, these genes may be used in the investigation of hepatotoxicity for better understanding of its mechanism.

Poster Abstracts

P24: Involvement of constitutive androstane receptor (CAR) in chemical-induced liver hypertrophy and carcinogenicity in mice

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It is critical in risk assessment of chemicals to clarify the relationship between liver hypertrophy and hepatocarcinogenicity. It has been well documented that the mechanism underlying phenobarbital (PB)-induced liver hypertrophy and carcinogenicity in rodents is associated with the induction of CYP2B via constitutive androstane receptor (CAR). The present study was aimed to elucidate the involvement of CAR in the mechanisms of liver hypertrophy and/or carcinogenicity induced by chemicals using CAR-knockout mice.

In Experiment 1, 6-week-old male CAR-knockout mice or wild C3H mice were given piperonyl butoxide (PBO), decabromodiphenyl ether (DBDE) and PB at a dose of 5,000, 50,000 and 500 ppm in their diet, respectively. Control groups received basal diet alone. After 4 weeks, livers were weighed and liver tissues were analyzed by histopathological examination, immunohistochemistry (CYP2b10) and molecular biological examination (*Cyp1* family, *Cyp2b* etc). The results showed that the induction of Cyp2b10 protein and gene found in all treated groups of wild mice was suppressed in the DBDE- or PB-treated groups but little affected in the PBO-treated group of CAR-knockout mice, suggesting that PBO-induced liver hypertrophy would not be induced via CAR. It was also concluded that DBDE induces liver hypertrophy via CAR, but the induction pattern of CYP1 family is different from PB.

In Experiment 2, 5-week-old male CAR-knockout mice or wild C3H mice were injected intraperitoneally with diethylnitrosamine (DEN). After 1 week, PBO, DBDE or PB was fed for 26 weeks at a dose similar to Experiment 1. Livers were examined histopathologically for the presence of preneoplastic and neoplastic lesions. In wild mice, neoplastic lesions were increased in all treated groups, the PBO-treated group being the most remarkable. In CAR-knockout mice, neoplastic lesions were scarcely developed with PB, dramatically reduced with PBO and somewhat reduced with DBDE without statistical significance as compared with wild mice. These data suggest that the mechanisms of liver carcinogenicity induced by test chemicals are more or less mediated by CAR, but different in process from those of liver hypertrophy.

Poster Abstracts

P25: Toxicopathological effects of chlorpyrifos on the lung, brain and liver of rabbits with dermal exposure

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Chlorpyrifos, a well known organo-phosphate insecticide, is widely used all over the world for agriculture production. This research aimed to evaluate adverse effects in the lung, brain and liver of rabbits induced by dermal exposure to chlorpyrifos, based on clinical pathology and histopathological changes. Twelve rabbits were divided randomly in two groups of six animals, i.e., a treatment and a control group. The treatment group was given chlorpyrifos via dermal contact at the doses of 50, 100, 250 and 400 mg/kg body weight for a period of 28 days. The control group received ethyl alcohol, the solvent of chlorpyrifos, with the same method. Blood samples from marginal ear vein of all animals were collected on days 0, 7, 14, 21 and 28. By using the statistical T-Test, the data obtained suggested that chlorpyrifos had harmful effects on the hematological and biochemical indices in the treatment group. A significant increase in the level of ALP, AST, ALT and cholesterol in the serum ($p < 0.05$), ($p < 0.01$) was observed. A significant decrease was observed in triglyceride level, total serum protein level, red and white blood cell counts and hemoglobin (Hb) in the treatment group, compared to control group ($p < 0.05$). Microscopical investigations on treatment group revealed emphysema, alveolar epithelialization, infiltration with lymphocytes and interstitial pneumonia in the lungs. Pyknosis of hepatocytes occurred in the liver, and necrotizing and hemorrhagic encephalitis (perivascular lymphoid cuffing), hemorrhage, edema, meningitis, necrosis of Purkinje cells were observed in the cerebellum, and focal gliosis was seen in the brain. According to the results of the present study, we conclude that dermal exposure with chlorpyrifos has toxic effects on biochemical and hematological parameters and can cause pathological changes in the lung, brain and liver.

Poster Abstracts

P26: Alteration of toxic-related gene expression of fluorescent whitener in vivo

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7-Diethylamino-4-methylcoumarin (DEMC) is one of the fluorescent whiteners (FWA) which are chemicals added to most fabrics and papers during manufacturing to increase color temperature, 'whiteness', and 'brightness'. There are other kinds of FWAs which are N-methyl-4-methoxynaphthalimide (MMNI) or VBL. There are some studies about DEMC's protective effects of the biological activity but there are few papers about toxicity of DEMC *in vivo* experiments. Zebrafish (*Danio rerio*) are an appropriate vertebrate animal model in aquatic environment. In this study, we used wild type zebrafish embryos at 3.0 days post fertilization (dpf). Test solution with DEMC concentrations were negative control (without vehicle), 0 (with vehicle: 0.01% v/v ethanol), 0.25, 0.5, 0.75, 1.0, 1.25, 1.5 and 2 ppm. DEMC was dissolved in 100% ethanol and the final ethanol concentration in each test solution was below 0.01%. We investigated survival rate, hatching rate, heart rate and morphological changes due to toxic effects. Quantitative RT-PCR was performed on these samples to determine the expression of different genes related to apoptosis, cell cycle arrest, mitochondrial metabolism and oxidative stress.

Poster Abstracts

P27: Toxicological and pathological study of the anticoccidial drug Dadcox (2.5% Toltrazuril)

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The purpose of this study is

- a) to determine the oral Median Lethal Dose (LD₅₀),
- b) to determine the repeated oral dose toxicity (subchronic), and
- c) to investigate the dermal toxicity of the DADCOX 2.5% (Toltrazuril 2.5%),

an anticoccidial medication, in adult Sprague-Dawley rats (150-200g BW), and to report the clinical, body and organ weights, and pathological findings. Toltrazuril is widely used in chickens, turkeys and pigs for the prevention and treatment of coccidiosis, by administration via drinking water. Analysis of the results revealed that the LD₅₀ of DADCOX 2.5% is between 1000 to 1250mg/kg BW which is in agreement to what is reported in the literature (1000mg to 5000mg/kg BW). The rats were very quiet within 90-120 minutes from the start of the study. They gathered close to each other and were not interested to move. Their eyes were semi-closed and they were depressed and extending their heads forward suggesting some problems in respiration. Their muscles were weak and they could not stand on their feet. Convulsions and rolling on their back were not observed. The animals had very severe loss of appetite and decreased feed consumption. In the subchronic study where the tested materials were administered orally for 21 days, there was no significant difference in the mean body weights or internal organs (liver, spleen, kidneys, lungs, hearts and testes), between the treated (1:50 of the LD₅₀) and control groups (n=12 rats in each group). Gross pathological examinations of the different organs revealed no significant difference between the control and treated groups. Histopathological examinations revealed mild hydropic and some fatty degeneration in the liver, and mild congestion in the kidneys. In addition, there was no significant difference in the plasma total protein concentration or in the liver enzymes between the control and treated groups (Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT)). There was also no significant difference in the mean values of the Red Blood Cells (RBC) and White Blood Cells (WBC). When DADCOX 2.5% was applied on the shaved skin of the back of the rats, at a concentration of 20 mg/kg BW for 21 days, it was not irritating and there was no mortality among the treated rats. Slight reversible vacuolar hepatocytes degeneration was present in some treated rats and there was a very mild effect on the kidneys. Histopathological examination of the skin revealed no substantial changes. Thus, we consider DADCOX 2.5% a safe anticoccidia medication, with no clinical toxic effects when administered orally to rats for 21 days.

Poster Abstracts

P28: An investigation on the hepatogenic poisoning by garlic tablet prescription in comparison with clofibrate drug in hyperlipemic rats

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Hyperlipidemia is one of the most effective and dangerous known factors in cardiovascular diseases. Increasing interest in modern medicine for herbal drugs, which seem to have fewer side effects, had led to do this investigation.

A comparison between two drugs, clofibrate and garlic tablet, both known to decrease blood cholesterol levels, was performed in order to assess any variation in the hepatic enzyme activity in rats.

The investigation was done on 28 mature male rats weighing 250 ± 10 g. These rats were divided into four groups:

- Control group: 14 days adaptation, then 60 days feed with normal diet, then blood tests.
- Hyperlipemic group: 14 days adaptation, 60 days feed fat saturated diet, then blood tests.
- Group under treatment with garlic tablets: 14 days adaptation, 30 days fat saturated diet, 30 days fat diet with garlic tablet, then blood tests.
- Group under treatment of clofibrate: 14 days adaptation, 30 days fat saturated diet, 30 days fat diet with clofibrate, then blood tests.

Blood examination was done after heart puncture by chemical analysis and spectrophotometry in order to determine the level of liver enzymes such as SGOT, SGPT and ALP.

All results were examined with SPSS and ANOVA. The level of certainty in all tests was 95%.

The activity of hepatic enzymes such as SGOT, SGPT, ALP in the garlic treatment group, more decreased but clofibrate causes more increase in SGOT, SGPT and decrease in ALP activity.



Poster Abstracts

P29: Comparison of the effects of vitamin E and vitamin E-selenium on plasma markers of oxidation in cat nephrotoxicity

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Oxygen free radicals can be produced during the respiratory cell oxidation or by some chemicals. Oxygen active metabolites and lipidperoxidation play an important role in nephrotoxicity caused by oxidative agents such as aminoglycosides and their neutralization. In this respect, we determined

4 experimental groups of 5 (2-year old) male cats

Group 1: control (n=?)

Group 2: received gentamicin (20mg/kg, IM, 12h, 9 days)

Group 3: received vit E (400 IU, oral, qoD, 3weeks), then gentamicin (20 mg/kg, 12h, 9 days)

Group 4: received a combination of (vit E 400IU, oral, qoD+ se 2 mg/kg (b.w), IM, 3 weeks), followed by gentamicin as described above.

Blood samples were collected. The evaluation resulted in a decrease of blood Cr, BUN and urine protein (indicative as factors for renal tubular necrosis) and in a decrease of blood MDA and GPX activity. The total antioxidant capacity of plasma (TAC) was increased. [as lipid peroxidation markers] ($p < 0.05$) in group 4 rather than group 3. Then (Se-vit E) decrease GM- induced nephrotoxicity more than (vit E) in cats.

Poster Abstracts

P30: Vincristine toxicity effect on cerebellum formation of mice during pregnancy

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Introduction: Vincristine is alkaloid that was administered for inhibition of division of malignant tumor cells. Occurring of malformation in embryos was proved in pregnant mothers. However, there was no adequate information about its toxic effect in newborns cerebellum structures. Will considering to Blood Brain Barrier passing and cytotoxic effect, rate of destructive effects to formation of cerebellum in newborns was demonstrated.

Methods: In this study 20 female Mice were pregnant divided as two groups (control and experimental) accidentally. The experimental group received 3mg/kg in days 10 and 15 pregnancy (I.P). In end of pregnancy duration 48 newborns (control and experimental groups) were selected for histotechnique process and H&E staining then in continues considered under light microscope. It was used from T-test and SPSS software for analyzing data obtaining from quantities parameters.

Results: In base of morphologic observations performing , It was obtained significant decrease in weight, skull size and newborn growth ($P < 0.001$). In base of microscopic observations , cerebellum is seen such as primary formation. White matter of cerebellum was seen with decreasing in compaction of neuralgia cells accompany with deficiency in dismyelination of nervous fibers. Occurring of apoptosis was seen in epithelial cells of choroid plexus and in white matter neuralgia cells.

Conclusions: In base of obtaining resultants we can conclude that effects of anti-mitosis drugs can include inhibitive activity of drug to difference and proliferation of cortical cells of cerebellum and its formation ultimately and it causes to support of apoptosis induction in choroid plexus cells and cerebellum.



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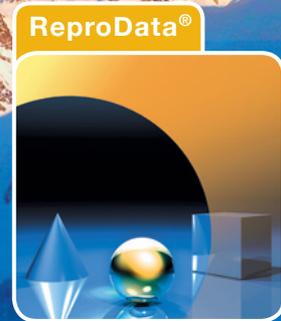
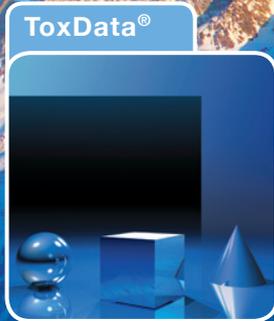




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