Welcome

Dear Colleagues and Friends

It is my great pleasure to welcome you to The Hague in The Netherlands for the 7th European Congress of Toxicologic Pathology.

The Hague is situated near the sea side and is a truely international city, holding international organizations such as Shell headquarters, OPCW (Organization for the Prohibition of Chemical Weapons), International Court of Justice, International Criminal Court, embassies, and many others. The Hague is the residency of the Dutch Parliament and the Royal Family. Besides that, it features many parks and gardens as well as art galleries, museums and fine restaurants.

The ESTP Congress will take place in the wonderful venue of ''The Kurhaus''. In a world of increasing globalization, also among the Toxicologic Pathologists, this congress gives us the perfect opportunity to meet with participants coming from all over the world. The congress will start with an IATP program „Systemic Pathology – Whole Animal Responses to Toxicity" on Tuesday, September 15th. The subsequent program of the ESTP called: „Drug Induced Toxicologic Pathology – Examples and Methods Used", deals with induced lesions of several organs, presented by the most eloquent and profound speakers on the different relevant subjects.

This splendid program was organized thanks to the Scientific Committee with members and experienced experts on the field of Toxicologic Pathology from different countries. I am also delighted to present the annual BSTP Dr.Chirukandath Gopinath Lecture named for one of the most influential and well liked practitioners in our profession. On Thursday, 17th September, Dr Gopinath will introduce Dr Bob Maronpot as key note speaker on xenobiotic induced liver lesions. The BSTP are generously funding this lecture for this and future congresses.
The welcome reception sponsored by the Mayor of The Hague, will take place Wednesday 16th in the Town Hall. Busses with departure from the Hotel Kurhaus will take us there with a special program.

I look forward to meeting you all on Thursday, September 17th in the evening at „Restaurant Doen" which will provide us with an excellent dinner and ambiance as well as the well known dance floor for those who enjoy not only sitting behind the microscope but dynamically enjoy music in a pleasant environment.

I would like to thank our organizers, helpers, speakers, partners, exhibitors and sponsors. Thank you all for coming to the 7th European Congress of Toxicologic Pathology.
We wish you all a warm welcome to ''The Hague'' and hope you enjoy both the scientific meeting as well as the conference's social program in a pleasant way along the Dutch coast.

Bob Thoolen
Local Organizer, ESTP

Table of Contents

Welcome 1

Table of Contents 2

General Information 5

Local Organizing Committee 5
Scientific Organizing Committee 5
IATP Organizing Committee 5
ESTP Congress Venue (Site Map) 6
Congress Venue 7
Accessibility for Persons with Disability 7
Access to the congress venue from Airport Amsterdam-Schiphol 7
Access to the congress venue from Airport Rotterdam 7
Access to the congress venue from Main station Den Haag 7
Parking 7
Taxi Scheveningen 8
Climate 8
Registration Desk 8
Speaker Information 8
Poster Presentation 8
Language 9
Internet Access 9
Messages 9
Congress Bags 9
Gastronomy 9
Safety and Security 9
Emergency Calls 9
ESTP Board Meeting 10
ESTP General Assembly 10
IATP ad hoc Committee 10
IFSTP EC-Meeting 10
ESTP Slide Seminar 10
Congress CD-ROM 10
Abstract Publication 10
ESTP Publication Award 11
Social Events 11
Industry Exhibition 11
Thanks to our Exhibitors 12
Thanks to our Sponsors 13

Table of Contents

Program – Satellite Symposium 14
Tuesday September 15, 2009 14

Congress Program 15
Wednesday September 16, 2009 15
Thursday September 17, 2009 17
Friday September 18, 2009 18

Speaker Abstracts 19
S1: Kinase Inhibitors in Oncology – Unexpected Cardiovascular Toxicity in Preclinical Studies 19
S2: Overview of drug-induced vasculopathy in laboratory animals 20
S3: Toxicologic pathology aspects of chemically induced cardiovascular toxicity, as seen in National Toxicology Program (NTP) studies 22
S4: The Nose-Brain Connection: An Introduction 23
S5: MRI and PET Scan of the Brain 24
S6: Neuroinflammation and altered brain immune responses upon inhalation of particles and gases 25
The BSTP Chirukandath Gopinath Lecture
S7: Xenobiotic-induced Toxicologic Pathology of the Liver 26
S8: Hepatocyte death: using toxicity for therapeutic options 27
S9: Xenobiotic-induced Rodent Tumors of Questionable Relevance to Human Cancer Risk 28
S10: Experiences of an external consultant – Successes & Failures 29
S11: Drug-Induced Phospholipidosis 30
S12: Cynomolgus monkey as animal model in toxicologic pathology 31
S13: Animal skin models and relevance for man 32
S14: Toxic responses of the skin 33
S15: Subcutaneous tumour issue with many of the PPARS 34

Slide Seminar Abstracts 35
Case 1: Renal papillary eosinophilic droplets in female rats treated with a novel compound 35
Case 2: Spontaneous hypertrophic and/or hyperplastic renal lesions with cellular atypia in young CD1 mice 37
Case 3: Pulmonary Vascular Lesion in a Mouse 38
Case 4: Arterial mineralization in the wall of the aortic arch in the dog 40
Case 5: Histological evidence of epitheliotropic lymphoproliferative disease in a zebrafish 41
Case 6: A case of pseudo-placentational endometrial hyperplasia in a Beagle dog 43
Case 7: Laser microdissection and quantitative real time PCR applied in characterization of IGF-1- and insulin receptor mRNA expression in the rat mammary gland 45
Case 8: Unexpected Nasal Changes after Oral Gavage in Rats and the Relation to Delayed Gastric Emptying 47

Table of Contents

Poster Abstracts 49

P1: Reactive vascular proliferative changes in the mesenteric lymph nodes of aging CD 1 mice 49
P2: Lead nitrate exposure induces structural changes but not the DNA fragmentation in the rat liver 50
P3: Identification of new structural alerts for hepatotoxicity by cluster analysis of a human adverse effects data set 51
P4: Hepatoprotective effect of Curcuma longa L. in D-galactosamine induced liver injury in mice: Evidence of antioxidant activity 52
P5: Effects in CD-1 mice liver following prenatal DEHP exposure 53
P6: Air pollution, heart inflammatory signaling molecules and chocolate 54
P7: Bleomycin-induced lung fibrosis model in the mouse 55
P8: Index values in the differential bone marrow count in Sprague-Dawley rats: available tools to evaluate haematotoxicity 56
P9: Toxicity effect of vincristine –treatment on mice testis tissue 57
P10: Predicting rat carcinogenicity findings using chronic study results 58
P11: Spontaneous multiple intestinal neoplasia in B6C3F1 mice 59
P12: Cardiovascular effects in rats following exposure to a receptor tyrosine kinase inhibitor 60
P13: Quantifying Huntingtin protein aggregates in a transgenic mouse model of Huntington's Disease 61
P14: Evaluation of automated histology pattern recognition tool for selection and analysis of tumor regions in breast sections stained with ER, PR and Ki67 62
P15: Validation of immunohistochemical staining, concerning either markers for research use or monoclonal antibodies for therapeutic purpose, according to the Good Laboratory Practice principles at CIT 63
P16: Next generation virtual slide based workflows for toxicological pathology 64
P17: Intra-cardiac ectopic thyroid in a Han Wistar Rat: Case report and review of the literature 65
P18: Protective effect of Nigella Sativa seeds extract on CCl4-induced hepatotoxicity in albino rats (toxicological, biochemical and histological studies) 66
P19: The protective effect of Silymarin on the adrenal gland in chlorpyrifos intoxicated rats 67

General Information

Local Organizing Committee

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Bob Thoolen, Johannes Harleman, Marie-France Perron Lepage, Frieke Kuper, Eveline de Rijk, Paul-Georg Germann, Matthias Rinke, Noel Downes

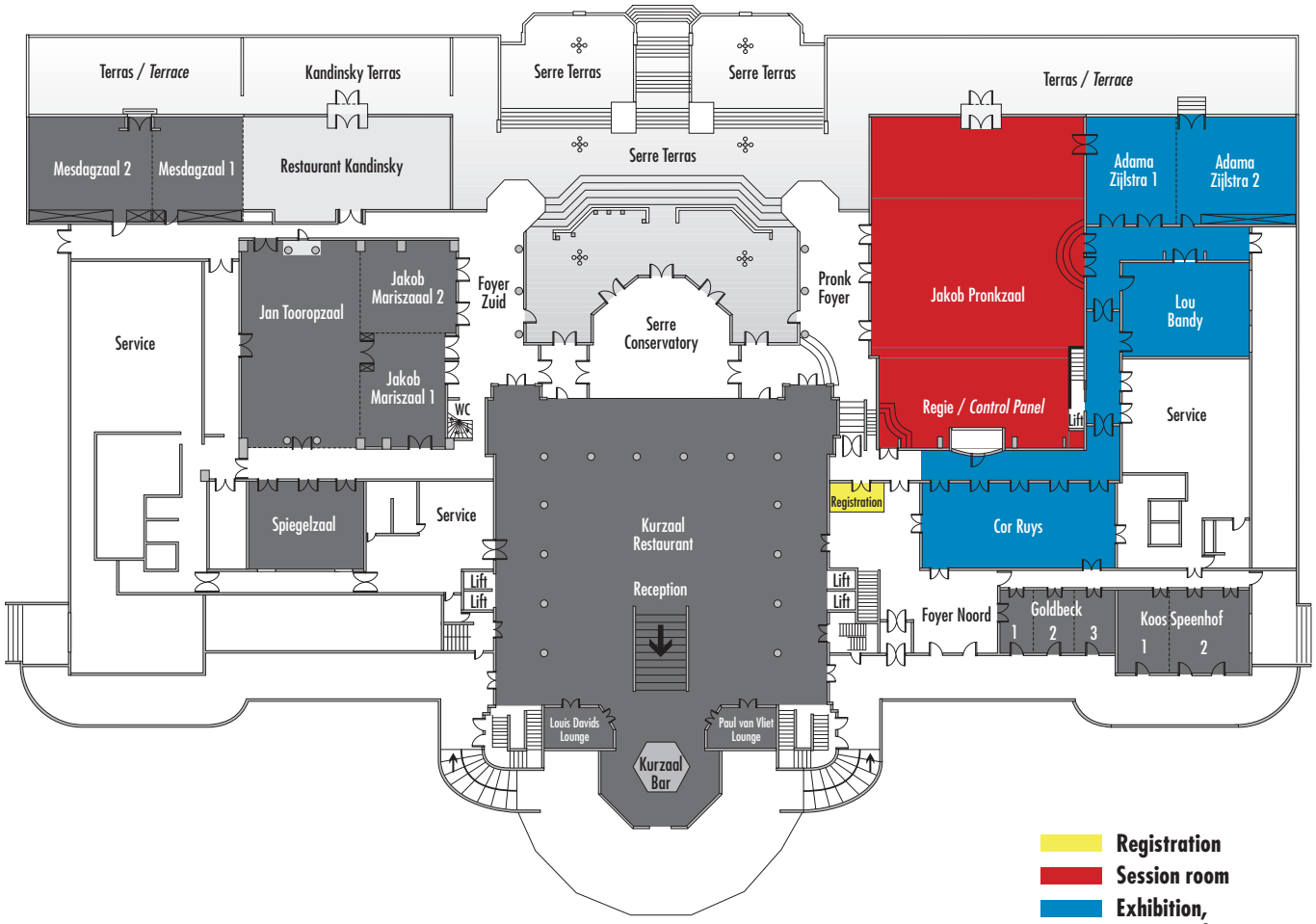
IATP Organizing Committee

Eugenia Floyd & Jerry Hardisty

General Information

ESTP Congress Venue (Site Map)

STEIGENBERGER
KURHAUS HOTEL
DEN HAAG



General Information

Congress Venue

Steigenberger Kurhaus Hotel
Gevers Deynootplein 30
2586 CK Den Haag-Scheveningen
Netherlands
Phone +31 70 4162636
Fax +31 70 4162646
Input GPS: Gevers Deynootplein

Accessibility for Persons with Disability

Please use the front door

Access to the congress venue from Airport Amsterdam-Schiphol

Onwards by train to main station Den Haag (Den Haag Centraal).
Please visit the website (www.eurotoxpath.org) for more information.
Taxi: At the Schiphol Plaza you can find taxis all the time
Taxi fare from Airport **Amsterdam-Schiphol** Den Haag Scheveningen 67,00 €

Access to the congress venue from Airport Rotterdam

Taxi: At Rotterdam Airport taxis can be found directly next to the terminal. For travelling with a taxi from and to Rotterdam Airport you can contact the Rotterdam Airport Taxi via telephone number +31 (0)10 - 262 04 06. All current credit cards (VISA, Mastercard, American Express, JCB, Diners Club and the international cabcharge card) are accepted.
Taxi fare from Rotterdam Airport --› Den Haag Scheveningen 55,00 €

Access to the congress venue from Main station Den Haag

Onwards by city train N° to Scheveningen/Kurhaus. Train station Den Haag Holland Spoor: Onwards with city train N° 1 to Scheveningen/Kurhaus.

Distances from the congress venue to:

City train station: at the hotel
Taxi stand: at the hotel
Main station Den Haag: 3 km
Motorway (A4): 5 km
Airport Rotterdam: 25 km
Airport Amsterdam: 40 km
City center Den Haag: 3 km

Parking

Public garage (P1)
Parking lot

General Information

Taxi Scheveningen

You can order taxi by special rates:

Train stations
Main station --› Kurhaus Hotel: € 18,–
Hollands Spoor --› Kurhaus Hotel: € 21,–

Kurhaus Hotel --› Main station: € 18,–
Kurhaus Hotel --› Hollands Spoor: € 21,–

Airport
Kurhaus Hotel --› Amsterdam Airport: € 67,–
Kurhaus Hotel --› Rotterdam Airport: € 55,–

Payment is possible by cash or by credit card.
Credit card payment needs to be mentioned when the taxi is booked.
Booking code ESTP 2009
The Call Center is reachable 24 hours a day, 7 days a week

The international number is: ++31703907722 or 0031703907722
The national number is: 0703907722

Climate

A temperate maritime climate: · winter daytime 0 – 10°c · winter nighttime often below freezing · summer daytime generally between 20 – 30°c. · summer nights tend to fall in the 10 – 20° range. Rainfall throughout the year is evenly distributed, often as a light, persistent drizzle. From March to May, the rain tends to fall in short, sharp bursts. The summer months can be quite humid, particularly in the warmest months of June to September. Sunshine is prevalent throughout May to August.

Registration Desk

The desk will be located at the foyer of the main session room (Jakob Pronk room). All 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:
Tuesday September 15, 11:00 – 18:00 h
Wednesday, September 16, 08:00 – 18:00 h
Thursday, September 17, 08:00 – 17:00 h
Friday, September 18, 07:30 – 14:00 h

Speaker Information

Video beamer, PC and overhead projector 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

(The poster area is kindly provided by TNO)

Posters will be exhibited during the entire Congress. Poster Sessions are scheduled for the lunch break. Authors therefore are kindly requested to be at their posters during the lunch break time to answer eventual questions.

General Information

Language

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

Internet Access

(The internet access is kindly provided by GSK)

A laptop with internet access is located in the Exhibition Area for service during the business hours. In case you want to use your own computer, wireless Internet access is also available via WLAN.

Messages

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

Congress Bags

(Congress bags were kindly provided by Global Pathology Support)

Gastronomy

Coffee, tea, refreshment beverage and pastries are served during the coffee breaks
Lunch is provided during the lunch breaks on:

Tuedsay, September 15 (only coffee break)
Wednesday, September 16
Thursday, September 17
Friday, September 18

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

Ambulance: 112
Fire brigade: 112
Police: 112

Police
For non-emergency police assistance anywhere in the Netherlands, please dial 0900 8844.
You will be connected to the nearest police station.

General Information

ESTP Board Meeting

An ESTP Board meeting will be held on Tuesday, September 15, 2009 at 18:15 h
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 Thursday, September 17 from 16:45 h – 18:45 h in the main session room (Jakob Pronk room)

IATP ad hoc Committee

The meeting will be held on Wednesday, September 16 from 13:00 h – 15:00 h in the "Koos Speenhoff I" room

IFSTP EC-Meeting

The meeting will be held on Thursday, September 17 from 13:30 h – 17:00 h in the "Koos Speenhoff I" room

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.eurotoxpath.org. The contributors will give presentations of their cases during the congress.

There are three slide sets available for microscopy during the congress.

Show your Slide to a Colleague

Microscopes and projection screen will be available on site at the exhibition area for Individual glass slide discussion on the "Show your slideto a colleague" stand of Zeiss. This gives the opportunity for everybody to bring interesting slides and discuss them with colleagues taking into account the GLP guidelines and/or management approval when appropriate.

Congress CD-ROM

A CD-ROM containing summaries and several presentations given at this congress (lectures and case reports) 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 2009 or early 2010.

General Information

ESTP Publication Award

The ESTP publication award is (kindly provided by Novartis Pharma AG) NOVARTIS
The award ceremony is scheduled for Wednesday Morning at the beginning of the Congress. Please, participate.

Social Events

Wednesday September 16, 2009
19:30 h Welcome Reception at the Town Hall The Hague.
(kindly sponsored by "City of THe Hague")

We are leaving at 17:30 h by a shuttle bus from the Kurhaus Hotel. A two hours sight seeing tour will bring you to the Town Hall.

Thursday, September 17, 19:15 h – late: Kindly supported by 3DHISTECH and AstraZeneca
ESTP Beach Party – only a 10 minutes walk from the Kurhaus Hotel in front of the sea. Detailed directions can be obtained at the Counter.

Strandclub Doen!

A snow white interior with hints of sand colors, a tight design and meeting in a characteristic environment. That is what beach club Doen! is.

Strandweg 9, 2586 JK Scheveningen

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 16, at 10:00 h and will then follow the same schedule as the conference.
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.
Please, visit the booths of our exhibitors.

General Information

Thanks to our Exhibitors

The ESTP greatly values the support from the following Exhibitors

3DHISTECH KFT.: www.3DHISTECH.com

Aperio Technologies: www.aperio.com

AstraZeneca: www.astrazeneca.com

Charles River Laboratories: www.criver.com

CIT: www.citox.com

Hamamatsu: www.hamamatsu.com

Instem: www.instem-lss.com

PDS Pathology Data Systems Ltd.: www.pds-europe.com

Propath Histopathology UK Ltd.

SlidePath LTD: www.slidepath.com

Ventana Medical Systems SA, a member of the Roche Group: www.ventanamed.com

Xybion Medical Systems: www.xybion.com/

General Information

Thanks to our Sponsors

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3DHISTECH KFT.: www.3DHISTECH.com

AstraZeneca: www.astrazeneca.com

BSTP: www.bstp.org.uk

Carl Zeiss AG: www.zeiss.com

F. Hoffmann La Roche AG: www.roche.com

Global Pathology Support: www.gpstoxpath.com

GlaxoSmithKline: www.gsk.com

Instem: www.instem-lss.com w

J&J: www.jnj.com

LPT GmbH & Co. KG: www.lpt-pharm-tox.de

Novartis: www.novartis.ch

Novo Nordisk: www.novonordisk.com

Nycomed Pharma GmbH: www.nycomed.com

PDS Pathology Data Systems Ltd.: www.pds-europe.com

Schering-Plough: ww.schering-plough.com/

Solvay: www.solvay.com

TNO: www.tno.nl

Program – Satellite Symposium

Tuesday September 15, 2009

13.15	**IATP/IFSTP meeting**
	Systems Pathology: **Whole Animal Responses to Toxicity** *Chairs: Eugenia Floyd* *Jerry F. Hardisty*
13.15 – 13.20	**Introduction and Welcome** *Session Chairperson: Hans Harleman*
13.20 – 14.00	**Species Differences in the Acute Phase Response** *P. David Eckersall*
14.00 – 14.40	**Use of Live Cell and Tissue Biochemistry to Understand Mechanisms and Identify Biomarkers in Toxicologic Pathology** *Peter O'Brien*
14.40 – 15.20	**The Role of Toll-Like Receptors in Innate Immunity** *Emmanuel Schenck*
15.20 – 16.00	**Forecasting Cytokine Storms: Everything Old is New** *Christopher Horvath*
16.00 – 16.30	**Coffee Break**
16.30 – 17.15	**INHAND** *Wolfgang Kaufmann, Susanne Rittinghausen*

Congress Program

Wednesday September 16, 2009

08.40 – 08.45 **Introduction and Welcome**
Bob Thoolen

08.45 – 09.10 **Novartis ESTP Award Ceremony**
Thomas Nolte & Ingrid Sjögren

Session 1: Cardiovascular System
Chairperson: Marie-France Perron Lepage

09.15 – 10.00 **S1: Kinase Inhibitors in Oncology – Unexpected Cardiovascular Toxicity in Preclinical Studies**
Graham Betton

10.00 – 10.45 **S2: Overview of drug-induced vasculopathy in laboratory animals**
Jean-Loïc LeNet

10.45 – 11.15 **Coffee Break:**
"Show your slide to a colleague"

11.15 – 12.00 **S3: Toxicologic pathology aspects of chemically induced cardiovascular toxicity, as seen in National Toxicology Program (NTP) studies**
Abraham Nyska

12.00 – 13.00 **Case Presentations 1:**
Session Chairperson: Sibylle Gröters

12.00 – 12.15 **Case 1: Renal papillary eosinophilic droplets in female rats treated with a novel compound**
Franck Chanut

12.15 – 12.30 **Case 2: Spontaneous hypertrophic and/or hyperplastic renal lesions with cellular atypia in young CD1 mice**
Armelle Grevot

12.30 – 12.45 **Case 3: Pulmonary Vascular Lesion in a Mouse**
Monique Y. Wells

12.45 – 13.00 **Case 4: Arterial mineralization in the wall of the aortic arch in the dog**
Henrik Soeeborg

13.00 – 14.45 **Lunch**

Congress Program

Wednesday September 16, 2009

Session 2: The Brain-Nose connection

Session Chairperson: Frieke Kuper

14.45 – 15.30	**S4: The Nose-Brain Connection: An Introduction** *Frieke Kuper*
15.30 – 16.15	**S5: MRI and PET Scan of the Brain** *Didima de Groot*
16.15 – 16.45	**Coffee Break**
16.45 – 17.30	**S6: Neuroinflammation and altered brain immune responses upon inhalation of particles and gases** *Lilian Calderon*
17.30 – 19.30	**Departure by bus from "The Kurhaus"** **Welcome Reception in The Town Hall**

Congress Program

Thursday September 17, 2009

Session 3: Liver
Session Chairperson: Bob Thoolen

09.15 – 10.00 **The BSTP Chirukandath Gopinath Lecture**

S7: Xenobiotic-induced Toxicologic Pathology of the Liver
Bob Maronpot

10.00 – 10.45 **S8: Hepatocyte death: using toxicity for therapeutic options**
Albrecht Wendel

10.45 – 11.15 **Coffee Break**

11.15 – 12.00 **S9: Xenobiotic-induced Rodent Tumors of Questionable Relevance to Human Cancer Risk**
Bob Maronpot

12.00 – 13.30 **Lunch**

Session 4: Safety & Efficacy
Session Chairperson: Paul-Georg Germann

13.30 – 14.15 **S10: Experiences of an external consultant – Successes & Failures**
Chirukandath Gopinath

14.15 – 15.00 **S11: Drug-Induced Phospholipidosis**
Richard Haworth

15.00 – 15.30 **Coffee Break**

15.30 – 16.40 Case Presentations 2:
Session Chairperson: Sibylle Gröters

15.30 – 15.45 **Case 5: Histological evidence of epitheliotropic lymphoproliferative disease in a zebrafish**
Simon Kimpfler et al.

15.45 – 16.00 **Case 6: A case of pseudo-placentational endometrial hyperplasia in a Beagle dog**
Francesco Marchesi et al.

16.00 – 16.20 **Case 7: Laser microdissection and quantitative real time PCR applied in characterization of IGF-1- and insulin receptor mRNA expression in the rat mammary gland**
Henning Hvid et. al

16.20 – 16.40 **Case 8: Unexpected Nasal Changes after Oral Gavage in Rats and the Relation to Delayed Gastric Emptying**
Siegrid Damsch et al.

16.45 – 18.45 **ESTP Annual Assembly**

19.15 – late **Congress Dinner and ESTP Beach Party**

Congress Program

Friday September 18, 2009

Session 5: Skin and Soft tissues: Models used in Toxicologic Pathology

Chairperson: Noel Downes

08.30 – 09.15	**S12: Cynomolgus monkey as animal model in toxicologic pathology** *Eveline de Rijk*
09.15 – 10.00	**S13: Animal skin models and relevance for man** *Peter Greaves*
10.00 – 10.30	**Coffee Break**
10.30 – 11.15	**S14: Toxic responses of the skin** *Noel Downes*
11.15 – 12.00	**S15: Subcutaneous tumour issue with many of the PPARS** *Peter Greaves*
12.00 – 12.15	**Closing remarks** *Bob Thoolen*
12.15 –	**Lunch**

Speaker Abstracts

S1: Kinase Inhibitors in Oncology – Unexpected Cardiovascular Toxicity in Preclinical Studies

Graham Betton

Betton Toxpath Consulting LLP; Marsh Side Farm, Rushton Spencer Macclesfield, Cheshire, SK11 oSE, United Kingdom

Factors in myocardial toxicity

The heart endures a high metabolic activity with no rest periods. Myoglobin has a high oxygen exchange rate with mitochondrial respiration and anti-oxidants needed to protect organelles from free radical injury. Rapid polarisation / depolarisation cycles rely on ion channels and ATP. There are high calcium levels in the sarcoplasmic reticulum T-tubules and uncontrolled release can cause toxicity in cytosol.

Cardiomyocytes are terminally differentiated in adults with no stem cell reserve; healing is only possible by fibrosis.

Toxicity of kinase inhibitors used in targeted cancer treatment

Many small molecule and antibody kinase inhibitors directed at cells surface receptors and second messenger pathways have shown cardiovascular toxicity in toxicology studies and in cancer patients. In the clinic, these are readily monitorable by MRI left ventricular ejection fraction (LVEF) and circulating toxicity markers such as troponins and brain naturetic peptide (BNP).

Cancer patients are typically on complex combination therapies, including anthracyclines such as doxorubicin, which are well known for causing cardiotoxicity. As the myocardium cannot repair, this represents a serious risk for clinical trials with tough ICH monitoring.

It is important to identify whether cardiotoxicity is caused by a direct effect on the myocardium or via indirect mechanisms causing haemodynamic changes affecting myocardial coronary perfusion or oxygen transport.

Directly cardiotoxic compounds are related to a broad range of pharmacological mechanisms such as growth factor signalling inhibition, survival signalling, apoptosis induction, cell cycle signalling, morphogenesis signalling and inflammation signalling. Cytotoxic agents acting on myocardium include anthracyclines, allylamine, calcium ionophores, divalent cations and phospholipidosis. Arrhythmogenic compounds affecting cardiac contractility can secondarily affect cardiac perfusion.

Indirect cardiotoxicity is typically a consequence of vascular actions of anti-cancer compounds, e.g. with effects on vascular tone affect BP and coronary flow. Safety pharmacology data, including repeat dose studies in toxicology species, are essential to determine such effect and need telemetry to evaluate haemodynamics as transient hypo- or hypertension events will be missed by baseline ICH monitoring. Other indirect effects can be due to angiogenesis effects, haemostasis effects, bone marrow suppression, anaemias or haemoglobin defects leading to secondary ischaemic injury to the myocardium. Metabolic disorders, e.g. diabetes, hyperthyroidism, can also be important because the heart is highly dependent on glucose and lipid energy supply.

Vasoconstrictor inotropes, e.g. noradrenaline, vasopressin, act both on blood flow and also increasing oxygen demand. Inotrope vasodilators, e.g. PDE inhibitors, and vasodilators, e.g. potassium channel openers, endothelin receptor antagonists, cause cardiotoxic changes via indirect actions. Other indirect effects, e.g. pulmonary hypertension, altered blood volume e.g. PPARs should also be considered.

In conclusion, cancer chemotherapy using either traditional cytotoxic small molecules or newer targeted therapies with small molecules or antibodies need careful evaluation for cardiotoxic potential both in animals and in the clinic.

Speaker Abstracts

S2: Overview of drug-induced vasculopathy in laboratory animals

Jean-Loïc Le Net,
Le Net Pathology Consulting 18, Rue Henri Dunant , 37400 – Amboise Cédex, France

Drug-induced vascular injury is reported with increasing frequency in preclinical toxicity studies. Owing to the lack of reliable biomarkers and lack of thorough understanding of underlying pathogenic mechanisms, drug-induced vascular injuries have hindered the development of life saving therapies when the therapeutic index and/or safety margins are low. Although the vascular injuries recognized in laboratory animals following administration of drugs are not known to occur clinically, some of the mechanisms leading to vasculopathy preclinically may have some relevance to humans and they must therefore be fully characterized.

Mechanisms leading drugs to induce vascular injuries in laboratory animals include direct toxic effect, immune-mediated mechanisms and/or alteration of the hemodynamic forces (shear and/or hoop stress). Examples of direct acting vascular toxicants are allylamine, β-aminoproprionitrile, monocrotoline, some antiproliferative anticancer drugs and all irritant compounds at the injection sites. Drugs and biologics can activate the immune system which in turn can cause endothelial cell injury or modulate the endothelial cell in such a way as to trigger an inflammatory response and an immune attack directed against the vessel wall. The immune effectors mediating acute drug-induced vascular injury have not been extensively identified. Vasculopathy induces by vasoactive drugs have been best characterized.

Vasopressors such as vasopressin, 5-hydroxytryptamine, methoxamine, angiotensin, dopamine, adrenaline, noradrenaline or digoxin induce vascular medial necrosis and hemorrhage, involving mainly the small-sized arteries of many organs. A number of appetite suppressants such as aminorex fumarate, fenfluramine and dexfenfluramine have also been implicated in pulmonary hypertension in people.

Vasodilators induce vasculopathy (medial necrosis and hemorrhage) in coronary arteries of the dog and splanchnic vasculature of the rat, at predilection sites of spontaneously-occurring vascular diseases in these 2 species, namely coronary arteritis in dogs and polyarteritis nodosa in rats. The vasculopathy is linked to the pharmacologically induced systemic hypotension and / or localized increased blood flow. In rats, doses of PDE III inhibitors that induce splanchnic arterial lesions result in a decrease in arterial blood pressure by about 40% for up to 12 h and equipotent hypotensive doses of minoxidil, a potassium channel opener and SK&F 95654, a PDE III inhibitor, produce morphologically identical lesion. In dogs, the administration of minoxidil with glyburide, a potassium channel blocker, prevents minoxidil-induced coronary arterial lesions, irrespective of the plasma concentration of minoxidil, provided the minoxidil-induced hypotension and tachycardia are blocked or attenuated. In dogs, minoxidil and SB209670, an endothelin receptor antagonist, induce coronary arteriopathy in dogs. SB209670 does not significantly increase heart rate or decreases blood pressure however both compounds cause a 6-fold increase in coronary blood flow. The endothelial and the smooth muscle cells not only play an important physiological role in mediating vascular tone but the pharmacologic target is often located on these cells (K+ channels, cGMP-inhibitable phosphodiesterase, endothelin receptors, adenosine receptors ..) and the disproportionate distribution of endothelinB receptors in the right coronary arteries of dogs has been implicated in the endothelin receptor antagonist-induced coronary pathology in this species. Overall, the vascular toxicity is considered to be related to vasodilatation, increased blood flow, inability of the vascular beds to maintain tone, increased shear (endothelial cells) and hoop stress (vessel wall) leading to inter-endothelial breaks and gradual breakdown of vessel wall integrity.

The hemodynamic stresses caused by vasoactive compounds can potentiate the development and severity of spontaneous occurring immune mediated vasculitis. This has been observed in rats with a number of vasodilating agents that increase the incidence of polyarteritis nodosa and in dogs with phosphodiesterase inhibitors that increase the incidence / severity of spontaneous occurring arteritis in dogs even leading to the full clinical expression of idiopathic polyarteritis (Beagle pain) following administration of PDE V inhibitors.

Speaker Abstracts

The ideal biomarkers of vascular injury should be useful in both preclinical and clinical studies, specific and sensitive (the vasculopathy is not usually widespread), mechanistically linked to pathology, altered very early prior to morphological changes and return to baseline values when there is no further tissue damage. Some years ago, in the preclinical development of vasodilators such as potassium channel openers or phophodiesterase 3 inhibitors, there was a good correlation between occurrence of vascular / cardiac toxicity and changes in physiological biomarkers (decrease in mean blood pressure and reflex tachycardia). The monitoring of blood pressure and heart rate was therefore considered to be optimal surrogate markers of vascular injuries in clinical studies and the risk of inducing drug-induced vessels damage in hypertensive patients at doses tailored to reduce abnormal blood pressure to normal was considered to be low. The industry is now developing drugs that cause vascular injury in animals without changes in systemic blood pressure or heart rate (e.g., endothelin receptor antagonists, adenosine agonists or PDE 4 inhibitors) and huge research efforts are now directed towards the identification of reliable biomarkers. Drug-induced vascular injury is not primarily an inflammatory change and as such the specificity/sensitivity of acute phase proteins (e.g. CRP), adhesion molecules and other inflammatory markers are low. Biomarkers of endothelial cell injury or nitric oxide pathway (vWF, vWFpp, vascular endothelial growth factor, endothelin, caveolin-1, asymmetric dimethyl arginine, nitric oxide and circulating endothelial cells) have shown some values however more work is needed and the applications of new technologies (omics and flow cytometry) will undoubtedly aid in the identification of a unique panel of biomarkers useful for assessing not only early but also progressive vascular injury.

Overall, when drug-induced vascular injury is reported, the risk assessment must continue to be based on pharmacodynamic / pharmacokinetic / pharmacological activities of the compound, dose dependency, reversibility of changes, presence of vascular lesion in other species, appropriate in vivo biomarkers (cardiovascular parameters ….), therapeutic index and therapeutic indication leading to sound scientific clinical judgment on a case-by-case basis.

Speaker Abstracts

S3: Toxicologic pathology aspects of chemically induced cardiovascular toxicity, as seen in National Toxicology Program (NTP) studies

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Potential cardiovascular toxicity of environmental agents and pharmaceuticals poses a major concern to health and regulatory authorities. Epidemiological studies have associated cardiovascular and respiratory morbidity and mortality with particulate matter (PM) air pollution, particularly in susceptible humans with concurrent cardiovascular and pulmonary diseases. Between 1961 and 1992, 131 drug products were withdrawn from the markets in Europe and the US. Ten of the 131 were withdrawn as the result of cardiovascular toxicity.

Studies in laboratory rodents are used to investigate the potential toxicity of various agents, identification and characterization of lesions suggesting cardiotoxicity are vital. Morphologic criteria have been described for degenerative myocardial lesions in rodents, but even with these criteria, differentiation of spontaneous from toxicity-induced lesions may be difficult. The histopathological pattern of lesion development may help determine whether the myocardial damage is due to injury of the coronary vasculature (in which case lesions tend to be multifocal) or due to direct myocardial cell toxicity (in which case lesions tend to affect much or all of the myocardium diffusely). In view of this observation, a retrospective light microscopic evaluation was performed on the hearts of F344 rats and B6C3F1 mice from National Toxicology Program (NTP) studies on six chemicals that produced myocardial toxicity in order to provide a detailed morphologic characterization of spontaneous versus treatment-induced myocardial lesions (Jokinen et al, Cardiovasc. Toxicol. 5:227-244, 2005). The findings at light microscopic evaluation, particularly when taken in conjunction with the results of other techniques, such as ultrastructural examination and special staining, may give a general indication of the potential mechanism of cardiotoxicity, and suggest possible areas for mechanistic studies to define more clearly the actual mechanism of toxicity. The lecture will present an overview of the morphologic aspects associated with chemically and drug-induced cardiovascular toxicity, as seen in the NTP studies.

Speaker Abstracts

S4: The Nose-Brain Connection: An Introduction

C. Frieke Kuper

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The connections between the brain and nasal tissues and structures are manifold. First of all, the nose monitors, conditions and filters inspired air. It is the site of smell or olfaction, long considered to be a less importance sense than sight of hearing in humans. Four regions of olfactory sensory cells have been distinguished: the main olfactory epithelium, Organ of Jacobson/ vomeronasal organ, organ of Masera/septal organ and the Gruenenberg ganglion. The sensory cells of the four olfactory regions differ cytologically, detect different odor molecules, and their axons lead via the main or accessory olfactory bulbs to different parts in the brain. The main olfactory epithelium itself is divided in zones that are defined by the expression of odorant receptors on sensory cells that connect with glomeruli in a corresponding zone of the olfactory bulb. Although the exact nature of the chemoperception by the various olfactory regions is not fully known, it is clear that smell has profound influence on neuroendocrine function and behaviour, among which sexual behaviour. Interestingly, nerve cells in the brain that control fertility have an olfactory origin. These gonadotrophin neurons have migrated from the nose to the brain during early foetal life.

Chemoperception other than olfaction is dominated by an extensive, intraepithelial, network of trigeminal nerve branches. Exposure of the nasal mucosa to irritants elicits respiratory, cardiovascular and hormonal responses that may protect the lower respiratory tract against these compounds. The fine, unmyelinated trigeminal nerve endings of the trigeminal nerve branches, and solitary epithelial chemoreceptor/sensory cells are most likely mediators of these responses. In addition to a sensory function, the free nerve endings may have an efferent function such as control of ciliary motion and mucus secretion.

Nasal passages have an important function in drainage of the cerebrospinal fluid (CSF) and the interstitial fluid (ISF) of the brain, especially in small and/or young animals. The fluids drain along cranial and extracranial spaces of which the olfactory nerve is the most important. Substances and immune cells drainingvia the olfactory pathway can be cleared from the nasal submucosa by passage into terminal lymphatics and then to the cervical lymph nodes and bloodvasculature.

What are the implications of these nose-brain connections for toxicology and drug safety and efficacy? The olfactory pathway can be used to deliver drugs to the brain, but the pathway is also a route for hazardous substances to enter the brain thereby circumventing the blood-brain barrier. Some forms of dementia might be induced by damage to the brain via inhaled substances. In addition, brain and nasal local immune systems appear heavily intertwined and it will not be easy to unravel these. The challenge for the future will be to explore how toxicologic pathologists should investigate the nose-brian interactions and which tools are available to assist in the investigations.

Speaker Abstracts

S5: MRI and PET Scan of the Brain

Didima De Groot[1], M Berk[3], M Bogaard[1], N. Cappaert[4], VJ De Groot[1],. A. Heerschap[3], F Kuper[1], N Jetten[1], R Nederlof[3], R Stierum[1], E Uitvlugt[2], A Veltien[3], B Voet[1], E De Vries[2], A Van Waarde[2], W Wadman[4], A Wolterbeek[1], M. Radonjic[1]

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To meet scientific (science-based research using adequate and sensitive tools), social (reduced and efficient animal use) and commercial (acceptable costs) requirements for future research on efficacy and safety of drugs, chemicals and food components, innovations in toxicology and pharmacology are required. Among these innovations, in vivo imaging certainly offers ways to study structure and function of target organs under normal and pathologic/toxicologic circumstances. Especially for the pathologist, this opens completely new areas of research for the pathologist not only studies organ and celstructures after death, but also in vivo during the evolvement of normal and pathologic morphology and histology.

Applications of in *vivo imaging*, i.e. the use of microPET, microCT, MRI, fMRI, MRImicroscopy and multimodality imaging offer an essential innovative tool in oncology, neuroscience, cardiology, stem cell therapy, the drug discovery process and toxicology. *In vivo* imaging is becoming more and more relevant and essential in reducing the gap between preclinical and clinical research. Disease processes can be followed *non-invasively* during the course of the disease.and so, more information of underlying mechanisms of *early onset* of the disease processes becomes available, allowing for *new development* of effective *therapeutic drugs*. Both *efficacy as well as the safety* of new drugs (but also functional foods/ food additives, or the safety of chemical exposure) can be tested at early onset and at different time-points during development of the disease process, drug treatment or chemical exposure. Imaging can provide unique objective surrogate clinical endpoints for intervention trials both in animals and in patients.

In recent years we have carried out a number of feasibility studies to test the relevance of *in vivo* imaging in safety pharmacology and developmental (neuro)toxicity studies. So far, we successfully applied [^{18}F] FDG micro-PET and Magnetic Resonance Imaging to study normal development of brain function and structure as well as the effects of well-known developmental neurotoxicants thereon [1, 2]. These *in vivo* imaging results were 'validated' with other conventional toxicology indicators (behavior and pathology) and more innovative technologies like toxicogenomics [3, 4], ex vivo hippocampal excitation [5], statistical modeling [5, 6] and stereology (neuron counts and brain region volume) in a rat study of developmental exposure to e.g. methylmercury [5].

To study neuronal damage or neuron loss longitudinally, we use *in vivo* Proton Magnetic Resonance Spectroscopic imaging (^{1}H-MRSI). In a study in rats prenatally exposed to ethanol neuron loss was demonstrated in the hippocampus, as reflected in the MRS spectrum by decreased *N*-acetyl (NA) to creatine (Cr) resonance intensity ratios.

In this presentation, we will share and highlight our experiences in *in vivo* imaging in the developing rat with special emphasis on the nervous system.

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Speaker Abstracts

S6: Neuroinflammation and altered brain immune responses upon inhalation of particles and gases

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BACKGROUND: Air pollution is a serious environmental problem. Mexico City (MC) residents are exposed to high and sustained year long concentrations of air pollutants including PM2.5 and endotoxins. MC children exposed to significant concentrations of air pollutants exhibit chronic respiratory inflammation, systemic inflammation, and adverse cardiovascular effects.

OBJECTIVES: The objective of this work is to review the neuropathological, brain structural, cognitive and innate immunity effects of chronic exposures to air pollutants upon clinically healthy children and young adults.

METHODS AND RESULTS: The objectives were accomplished through clinical studies in clinically healthy children, and two pathology studies: one in dogs and the other in brain samples from children and young adults with sudden accidental deaths. In the first clinical study we tested the hypothesis that exposure to severe air pollution plays a role on the immune responses of asymptomatic apparently healthy children. Blood measurements for markers of immune function, inflammatory mediators, and molecules interacting with the lipopolysaccharide recognition complex were obtained from two cohorts of matched children age (9.7±1.2 years) from MC (n = 66) and from a control city (n = 93) with criteria pollutant levels below current standards. MC children exhibited significant decreases in the numbers of natural killer cells (p=0.003) and increased numbers of mCD14+ monocytes (p ‹0.0001) and CD8+ cells (p=0.02). Lower concentrations of interferon γ (p= 0.009) and granulocyte-macrophage colony-stimulating factor (p=0.0002), an endotoxin tolerance-like state, systemic inflammation, and an anti-inflammatory response were present in the highly exposed children. In the second clinical study that included a comparative dog pathology study, children from MC (n: 53) and a low polluted city (n: 20), age 10.06 ± 2.18 y underwent psychometric testing and brain magnetic resonance imaging MRI. Seven healthy young MC dogs had brain MRI, measurement of mRNA abundance of cyclooxygenase-2, and interleukin 1 β in target brain areas. MC children exhibited significant deficits in a combination of fluid and crystallized cognition tasks, reflecting deficits in attention, concentration and short-term memory. Fifty-six % of MC children tested showed prefrontal white matter hyperintense lesions on MRI. Exposed dogs had frontal MRI lesions with vascular subcortical pathology associated with neuroinflammation. In the autopsy study we measured mRNA COX2, IL-1β, and CD14 in target brain regions from low (n: 12) or highly exposed residents (n: 35) 25.1 ± 1.5 years. Upregulation of COX2, IL-1β, and CD14 in olfactory bulb, frontal cortex, substantia nigrae and vagus nerves, disruption of the blood-brain-barrier, endothelial activation, oxidative stress, and inflammatory cell trafficking were seen in highly exposed subjects. Amyloid β42 immunoreactivity was observed in 58.8% of APOE 3/3 ‹25y, and 100% of the APOE 4 subjects, while α synuclein was seen in 23.5% of ‹25y subjects. Particulate material was seen in olfactory bulb neurons, and PM ‹100 nm in intraluminal erytrocytes from lung, frontal and trigeminal ganglia capillaries.

CONCLUSIONS: Sustained exposures to urban air pollution in children and young adults cause: 1. Immunodysregulation and systemic inflammation, 2. Cognitive deficits and brain structural changes, and 3. Neuroinflammation and neurodegeneration. The dysfunctional innate system plays a key role in the endotoxin tolerance-like state, the systemic inflammation, and the brain inflammatory and neurodegenerative responses, and exposure to air pollution should be considered a risk factor for Alzheimer and Parkinson's diseases. The impact of air pollution upon a developing brain with all their potential consequences ought to be of major public importance.

Speaker Abstracts

The BSTP Chirukandath Gopinath Lecture

S7: Xenobiotic-induced Toxicologic Pathology of the Liver

R. R. Maronpot

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Hazard identification for drugs as well as dietary and environmental exposures to xenobiotic agents is typically assessed in laboratory animals and may result in both adaptive and adverse effects on the hepatobiliary system. Typical non-neoplastic changes include cytologic alteration, hepatocellular hypertrophy/hyperplasia, necrosis, inflammation, and vascular changes. Proliferative effects from xenobiotic exposure include foci of cellular alteration, nodular hyperplasia, and hepatocellular neoplasia. Some degree of these hepatic toxicological effects also occur as spontaneous background lesions, can be exacerbated by treatment, and must be distinguished from direct effects of xenobiotic exposure. The association of foci of cellular alteration and hepatocellular tumor development, distinguishing cholangiofibrosis from cholangiocarcinoma, and predictivity of hepatocarcinogenic responses from prechronic study lesions will be explored.

Speaker Abstracts

S8: Hepatocyte death: using toxicity for therapeutic options

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Overactivation of apoptotic death receptors (DR) such as tumor necrosis factor receptor 1 (TNF-R1), CD95/Fas or tumor necrosis factor-related apoptosis-inducing ligand receptor (TRAIL-R) results in liver pathology, e.g. due to ethanol or viral exposure. Frequently, the ultimate consequence is development of hepatocelluar carcinoma (HCC). Here we report, how pharmacological intervention with DR-mediated apoptosis in the liver can be exploited to (i) target liver tumor tissue under simultaneous protection of healthy cells, or (ii) sensitize liver tumor cells for apoptotic immune-mediated eradication.

HCC is one of the most frequent cancers worldwide with poor outcome. TNF as the first immunological anti-tumor agent showed high efficiency, however, no selectivity between healthy and malignant tissue. We previously showed that hepatic ATP depletion by various phosphate-trapping ketohexoses such as fructose completely blocks TNF-induced hepatotoxicity in vitro and in vivo (J. Exp. Med. 191, 1975, 2000). Here we show that dedifferentiated hepatoma cells are lacking this genuine liver-typical fructose metabolising capability. Hence, upon fructose exposure, the intracellular ATP depletion and protection is impaired in malignant cells while primary intact cells are protected. We identified upregulation of hexokinase II as the biochemical mechanism which is responsible for the de-differentiated fructose metabolism in hepatoma cells. We demonstrate that tumor-specific changes of fructose metabolism modulate the protective effects of fructose against TNF-induced cell death and thereby introduce selectivity into immuno-pharmacological tumor therapy, where naïve intact cells are protected.

Histone deacetylases (HD) represent a novel target in cancer treatment. However, only few studies address the toxicological impact of HD inhibitors (HDIs) on malignantly transformed cells versus primary hepatocytes. We examined whether and how different classes of HDIs sensitise the human HCC cell line HepG2, primary healthy murine and human liver cells towards the death receptor agonists TNFα and CD95L. Apicidin, M344 (N-hydroxy-7-(-4-dimethylaminobenzol)aminoheptanamide), CBHA (m-carboxycinnamic acid bis-hydroxamide) and VPA (valproic acid) sensitized liver cell cultures towards CD95-triggered apoptosis with the following potency: Apicidin › M344 ≈ CBHA ›› VPA. Also in the intact organ, i.e. in the isolated perfused mouse liver, Apicidin sensitized towards CD95. No significant sensitisation towards TNFα was found in vitro. Western blot analysis showed that all HDIs examined downregulated the anti-apoptotic protein cFLIP, but only VPA affected additionally the expression level of XIAP. Furthermore, in models of the intrinsic apoptosis pathway, i.e. in HepG2 cells treated with Melphalan and in primary hepatocytes irradiated with UV light, exclusively VPA exhibited a significant sensitisation. These findings extend the biochemical and pharmacological basis for HDI therapy and provide an alert for clinical use in patients within an accompanying critical inflammatory state in which the CD95 system might be pre-activated.

Speaker Abstracts

S9: Xenobiotic-induced Rodent Tumors of Questionable Relevance to Human Cancer Risk

R. R. Maronpot
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Based upon over 50 years of conducting rodent bioassays for cancer hazard identification, it is apparent that only some xenobiotic-induced rodent tumors are potentially relevant to human cancer risk. Relative risk of cancer in tissues shared by both humans and rodents is based on the relevance of the mechanism of induction or the relevance of the mode of action of the xenobiotic in the rodent test system and whether that mechanism occurs in humans. Examples of rodent specific tumor induction of little relevance to humans include alpha-2-u globulin-related kidney tumors in male rats, urinary bladder tumors secondary to foreign body irritation, carcinoids associated with H2 antagonists, mammary gland neoplasms secondary to pituitary hormonal stimulation, and uterine endometrial cancer associated with dopamine agonists. Another category of xenobiotic-induced rodent tumors of uncertain relevance to human cancer risk includes tumors in the forestomach, Zymbal gland, Harderian gland, and preputial/clitoral gland. Marginal increases in tunica vaginalis mesotheliomas in male Fischer 344 rats are also of questionable relevance to human cancer risk.

Speaker Abstracts

S10: Experiences of an external consultant - Successes & Failures

Chirukandath Gopinath
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Consultants in toxicological pathology are mostly persons with considerable professional experience in the field, who have established themselves as experts or specialists. Usually their experiences of previous positions have earned them high reputations within the profession. Consultants work as independent sole traders or from an established company who may have other experts.

Those who seek out consultants are usually other pathologists, toxicologists, managers representing pharmaceutical, agrochemical or industrial chemical companies or contract research organisations.

The issues one is called to deal with vary widely and so do their complexities. Histopathology slide reviews for various reasons, such as confirming diagnoses, target organs, no effect levels, no adverse effect levels and peer reviews are among the services offered by consultants in this field. Other services include organising or participating pathology working groups, scientific advisory groups and production of expert reports.

Examples of issues dealt with as a consultant include reviews to discriminate reported treatment-related findings from artefacts/ physiological changes or age-related changes.
These also include expert reports to differentiate adaptive from adverse findings and adverse from expected production of pharmacologically mediated results or species-specific effects and to put the results in context using principles of mode of action and weight of evidence.
The presentation will illustrate these examples with data obtained from case studies.

Speaker Abstracts

S11: Drug-Induced Phospholipidosis

Richard Haworth
GlaxoSmithKline R&D, The Frythe, Welwyn, Hertfordshire, AL6 9AR, United Kingdom

Drug induced phospholipidosis (PLD) is defined morphologically as the drug induced appearance of intracellular accumulation of lamellar bodies in a tissue. This presentation will review the appearance of PLD at light and electron microscopy level. Immunohistochemistry using antibodies to lysosomal associated protein (LAMP-2) to identify vacuoles arising from lysosomes will be illustrated. In addition, current thinking on potential mechanisms and risk assessment of PLD will be reviewed. PLD, in isolation, is not considered a toxicity. Within a particular tissue, the clinical significance is determined by secondary damage or impaired function. Screening methods for candidate drug molecules, including in vitro and QSAR approaches will be discussed.

Speaker Abstracts

S12: Cynomolgus monkey as animal model in toxicologic pathology

E. de Rijk, S. Greijdanus, E. van Esch
Schering-Plough Corporation, Dept. TDD, Nistelrooisebaan 3, NL-5374 RE Schaijk, The Netherlands

The use of Old World primates in drug development and toxicological research has increased significantly within the last few years. One of the main reasons is their similarity to human beings with respect to anatomic and (patho) physiological characteristics. Although initially the rhesus monkey was studied frequently, the Crab-eating or long-tailed Macaque (*Macaca fascicularis*), also called the Cynomolgus monkey, have become most popular in both general toxicity studies and reprotoxicity studies.

Of course, there are several draw-backs in using monkeys as animal model within toxicity studies such as ethical issues, low number of animals per group, costs and availability of the animals, age of the animals, zoonoses, relatively high incidence of back-ground findings, different sources (free living and/or purpose-bred), high costs. In case the use of the monkey as the non-rodent species is unavoidable, the reasons above should urge the scientist to be sure to have a well-considered experimental design.

This presentation is not an attempt to cover a complete overview of histopathological changes that may occur in Cynomolgus monkeys within toxicology studies, but is primarily intended to give a few illustrative examples of drug-induced findings, background findings and issues that were encountered in this species the last few years within Schering-Plough (EU).

Particularly the female reproductive system of the Cynomolgus monkey has been proven to be very similar to human, since monkeys are the only mammals that, like women, have a comparable menstrual cycle with monthly bleeding. Especially testing of hormonal compounds in Cynomolgus females will in most cases give results that are most useful in extrapolation and human risk assessment. In the presentation, attention is given to the normal aspects of the Cynomolgus menstrual cycle and it will be compared to the menstrual cycle in human. This information is necessary to be able to distinguish normal background and hormonal cyclic changes from drug-induced (hormonal) changes. In addition, an example of a Cynomolgus-specific lesion will be given being either drug-related, a background finding or a normal physiological finding under certain conditions.

Besides the female reproductive system, also the reproductive system in Cynomolgus males is supposed to be very comparable to men. However, when the morphological structures of the testes were examined they appear to be quite different between Cynomolgus monkey and men. The question whether the Cynomolgus monkey is a good model to study drug (hormonal)-related effects on the male reproductive system will be addressed in the presentation by an illustration of the differences and examples of drug-related effects. Additionally, the problem of animal maturity/sexual maturation will be discussed.

Not only reproductive organs but also other organ systems within Cynomolgus monkey and human seem to respond similar to certain triggers. A good example is the occurrence of gastritis that is often observed in the treated animals within a toxicity study. Also an example of a rather rare lesion in the liver will be discussed with respect to the relevance for men together with a possible way to investigate the mechanism.

After years of experience in performing toxicity studies in Cynomolgus monkey, we believe that despite some serious draw-backs they are very useful in the evaluation of drugs with respect to the potential risk for men.
In the end.....we are not that different!

Speaker Abstracts

S13: Animal skin models and relevance for man

Peter Greaves

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The skin is one of the most common targets of adverse drug reactions and it has been estimated that they occur at a rate of 0.1 to 1% of treated patients in pre-marketing clinical trials[1] and in of 2-3% of patients in hospital settings[2]. Many of these reactions are benign exanthematous or maculo-papular eruptions often described as 'drug rashes'. However, some reactions are more severe and it has been estimated that 7% of the drugs withdrawn from the market or those requiring additional labelling warnings have been a result of skin reactions[3].

Most of drug reactions in the skin are believed to be due to different types of hypersensitivity reactions for which there are no reliable predictive animal models, at least for small molecules. Nevertheless a test for type IV, delayed-type or cell-mediated type hypersensitivity such as the Buehler assay or guinea pig maximisation test is usually recommended for preclinical testing of topical drugs[4].

Not all skin drug reactions are immune-mediated. Examples include photo-toxic reactions, changes to pigmentation, anticoagulant skin necrosis, damage to hairs and nails from antiproliferative drugs, lipodystrophy from anti-retroviral agents, and atrophy from corticosteroid therapy as well as various skin changes from therapy with other hormones. It is probable that many of these can be predicted from standard preclinical studies. This is consistent with comparative data that showed that animal preclinical studies predict less than 40% of drug skin reactions in clinical trials[5].

Other causes of localised adverse effects in the skin and subcutis are those that result from the injection of drugs or implantation of biomaterials. A considerable body of comparative data has shown that histological evaluation of local tissues at the sites of injection or implantation of these substances in preclinical studies is reasonably predictive of their irritancy potential in humans. However it has to be kept in mind that agents administered by the intradermal route may be more immunogenic, stimulating skin-homing effector cells by virtue of its important role in immune surveillance, features that may also facilitate response to vaccination[6,7]. Indeed, novel injected macromolecules that are not intended as vaccines represent a special case because there is a need for careful discrimination of their irritant potential from their possible immune-mediated or pharmacodynamic effects at injection sites.

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Speaker Abstracts

S14: Toxic responses of the skin

Noel Downes
Sequani Limited, Bromyard Road, Ledbury, Herefordshire, HR8 1LH, United Kingdom

Although as scientists in toxicological pathology our main focus is on animal pathology it is clear that there are large differences in human skin compared to many of our animal systems. In this presentation there will be an examination of the relevance of toxicity in lab species for man.

- Topic to be examined and discussed include

Structure and Function in Man
- Toxic Responses of Epithelial Cells After Systemic Administration
- Toxicity to Epithelial Cells of Topically Administered Substances
  – In vitro models
  – Animal models of human skin
- Mechanistic Models - Mouse models and skin painting
- Non-epithelial cells in the epidermis
  – Langerhans cells
  – Melanocytes and pigmentation changes
  – Merkel cells

In recognition of the questionable relevance of many animla models there will be a strong focus on toxicity in human skin

Speaker Abstracts

S15: Subcutaneous tumour issue with many of the PPARS

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Treatment of rodents with peroxisome proliferator activated receptor (PPAR) agonists for long periods has been associated with the development of increased numbers neoplasms in a number of different organs. Agonists of the PPARα isoform have long been associated with the development of hepatic tumours in rodents, although this does not reflect significant tumour risk for humans[1,2]. Common tumour types associated with PPARγ and mixed isoforms in rodent carcinogenicity studies are mesenchymal tumours, principally lipomas, liposarcomas and fibrosarcomas in rats and vascular tumours in mice[3]. The lipomas are histologically similar to those in controls. The liposarcomas and fibrosarcomas in treated rats are both characterised by the presence of interlacing bundles of spindle cells as well as pleomorphic cells but unlike fibrosarcomas the liposarcomas contain neoplastic lipoblasts. In treated mice, most of the proliferative vascular lesions consist of benign and malignant endothelial neoplasms classified as haemangiomas and haemangiosarcomas, similar in appearance to those found spontaneously in mice although angiomatous hyperplasia and angiolipomas also occur[4].

All these neoplasms occur in modest numbers in treated animals and develop during the latter part of 2-year carcinogenicity studies. This situation is substantially different from those sarcomas that arise following treatment of rodents with powerful carcinogens such as vinyl chloride and polycyclic aromatic hydrocarbons that in certain situations have been associated with tumorigenic risk in humans. In such cases highly malignant and metastatic tumours occur within periods of a few months.

Thus, the pathology and the evolution of mesenchymal neoplasms during long term treatment with high doses of PPAR agonists reflects the general pattern of tumour development associated with a number of pharmaceutical agents and implanted biomaterials in rodents which probably have little or no direct relevance to humans when used therapeutically. Like the liver neoplasms developing in rodents following long term treatment with PPARα agonists, mesenchymal tumours associated with the other PPAR isoforms are unlikely to reflect a significant human tumour risk.

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Slide Seminar Abstracts

Case 1: Renal papillary eosinophilic droplets in female rats treated with a novel compound

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ABSTRACT:	A 7-day oral repeat dose study was performed with a novel compound in Crl:CD(SD) rats. At the end of the treatment period the animals were sacrificed and a short tissue list was retained and formalin-fixed. We describe an infrequently reported finding in the renal papilla of top-dose females.
ANIMAL(S):	
Species, breed	Crl:CD(SD) Rat
Sex	Female
Age	11 weeks
Study type	7-Day Oral Toxicity Study
Treatment	Compound X
Clinical findings	None
Organ(s)	Kidney
Gross finding(s)	None
Staining	H&E
CONTRIBUTOR'S MORPHOLOGIC DIAGNOSIS:	Kidney, collecting ducts and papilla epithelium : Multifocal, moderate intracytoplasmic eosinophilic droplets.
CONTRIBUTOR'S DESCRIPTION AND COMMENTS:	4/4 top dose females presented in the kidney multifocal moderate eosinophilic droplets in the collecting ducts and papillary epithelium. There was no sign of cellular degeneration or necrosis. These droplets were PAS (Periodic Acid Schiff) positive and slightly CAB (Chromotrope Aniline Blue) positive. Electron microscopy showed that these droplets were consistent with secondary lysosomes with organelle debris. This finding is rarely reported in the literature. Previous reports involving various compounds tend to associate this change with potassium deficiency. However the mechanism is unclear. In this study the serum/potassium levels were lower in top dose animals, especially in males. The discrepancy between the low serum/potassium level and the renal finding (absent in male) is unknown. In the literature, females were reported before to be more affected than males.

Slide Seminar Abstracts

LITERATURE:	Renal papillary cytoplasmic granularity and potassium depletion induced by carbonic anhydrase inhibitors in rats. RA Owen, G Durand-Cavagna, S Molon-Noblot, C Boussiquet-Leroux, PH Berry, N Tonkonoh, CP Peter, and LR Gordon Toxicol Pathol, Sep 1993; 21(5): 449-55. The nephropathy of experimental potassium deficiency. An electron microscopic study. Mary K. MacDonald, M. S. Sabour, Anne T. Lambie, and J. S. Robson Q J Exp Physiol Cogn Med Sci , Jan 1962; 47: 262 - 272. Nephrotoxicity of a novel antineoplastic platinum complex, nedaplatin: a comparative study with cisplatin in rats. T Uehara, H Watanabe, F Itoh, S Inoue, H Koshida, M Nakamura, J Yamate, and T Maruyama Arch Toxicol, Aug 2005; 79(8): 451-60. Comparative analysis of gene expression between renal cortex and papilla in nedaplatin-induced nephrotoxicity in rats Takeki Uehara, Takako Miyoshi, Noriko Tsuchiya, Koichi Masuno, Manabu Okada, Satoshi Inoue, Mikinori Torii, Jyoji Yamate, and Toshiyuki Maruyama Human and Experimental Toxicology, Oct 2007; 26: 767 - 780.

Slide Seminar Abstracts

Case 2: Spontaneous hypertrophic and/or hyperplastic renal lesions with cellular atypia in young CD1 mice

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ABSTRACT:	Atypical tubular hypertrophy/hyperplasia were present in several control and treated young adult CD-1 mice aged 10-12 weeks, which were involved in several short term toxicity studies run at Novartis Basel in 2007 and 2008. The Crl:CD1 (Icr) mice were delivered from the same provider but obtained from different European countries. The breeder performed further investigation in the corporate colonies and the incidence of spontaneous renal microscopic abnormalities revealed to be >10% in the whole pool of animals.
ANIMAL(S):	
Species, breed	Mice, CD-1: Crl
Sex	males and females
Age	10-12 weeks
Study type	NA
Treatment	NA (spontaneous finding)
Clinical findings	NA
Organ(s)	Kidney
Gross finding(s)	NA
Staining	H&E
CONTRIBUTOR'S MORPHOLOGIC DIAGNOSIS:	Hyperplasia/hypertrophy, tubular, simple or atypical, mostly bilateral, minimal to slight
CONTRIBUTOR'S DESCRIPTION AND COMMENTS:	Spontaneous microscopic abnormalities were observed in renal tubules of CD-1 mice aged 10-12 weeks in several 2-week toxicity studies run at Novartis. Both sexes were affected; but the incidence was slightly higher in males. Microscopic findings including minimal to slight hypertrophy with or without hyperplasia, simple or atypical, mostly bilateral, were present mostly in the cortex of several control and treated animals. The studies comprised different test articles (administered per os) and different vehicles. The microscopic findings were not considered to be test article or vehicle related.
LITERATURE:	- Early Onset of Spontaneous Renal Preneoplastic and Neoplastic Lesions in Young Conventional Rats in Toxicity Studies -- Lanzoni et al_ 35 (4) 589 -- Toxicologic Pathology - Spontaneous Renal Tubular Hyperplastic and Neoplastic Lesions in Three Sprague-Dawley Rats from a 90-Day Toxicity Study -- Hall et al_ 35 (2) 233 -- Toxicologic Pathology
ADDITIONAL COMMENTS:	The microscopic lesions presented here are common in aged mice, but unusual in young animals. They were not considered treatment-related for several reasons: several control animals were affected at a comparable incidence; standard vehicles were used; no dose-dependency could be established; different test articles were tested in independent studies. No description of such findings could be found in the literature concerning the mouse, but several publications report similar findings in rats.

Slide Seminar Abstracts

Case 3: Pulmonary Vascular Lesion in a Mouse

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ABSTRACT:	A single, focal lesion in a pulmonary vessel was observed in a 17-week old female from a colony of heterozygous MnSOD (SOD2) mice. It consisted of a protrusion of amorphous to granular material covered by endothelium into the vessel lumen. Irregularly dispersed spindle-shaped cells were present within this material. There were no clinical signs and no gross lesions observed in the affected animal. Similar lesions have been previously reported in the pulmonary arteries of aging Han:NMRI, CBA, and C57BL mice. The change appears to be restricted to the lung. Inflammation is generally absent. In the current case report, the affected vessel was thin-walled, but did not contain striated muscle in the media. It was therefore interpreted to be a pulmonary artery.
Label on histoslides	Scan to be submitted when available
ANIMAL(S):	
Species, breed	Heterozygous MnSOD (SOD2) mouse (background strain C57BL/6; backcrossed with C57BL/6 mice)
Sex	Female
Age	17 weeks
Study type	Colony animal (no study performed)
Treatment	none
Clinical findings	none
Organ(s)	lung
Gross finding(s)	none
Staining	H&E; no special stains
CONTRIBUTOR'S MORPHOLOGIC DIAGNOSIS:	Intramural plaque, mild
CONTRIBUTOR'S DESCRIPTION AND COMMENTS:	The lesion consists of a lightly eosinophilic, amorphous to granular material covered by endothelium that protrudes into the lumen of a thin-walled pulmonary vessel. A number of spindloid cells are irregularly interspersed within this material, which appears to overlie the tunica media of the vessel. In one small area, the material appears to lie beneath the tunica media as well. Inflammation is not a feature of this lesion.

Slide Seminar Abstracts

LITERATURE:	Ernst H, Dungworth DL, Kamino K, Rittinghausen S, Mohr U (1996) Nonneoplastic Lesions in the Lungs. In: Mohr U, Dungworth DL, Capen CC, Carlton WW, Sundberg JP, Ward JM (eds) Pathobiology of the Aging Mouse Volume I. ILSI Press, Washington, D.C., pp. 281-300 Kay, JM (1992) Blood Vessels of the Lung. In: Parent RA. Treatise on Pulmonary Toxicology Volume I. Comparative Biology of the Normal Lung. CRC Press, Boca Raton, pp. 163-174 Rehm S, Wcislo A, Deerberg F (1985) Non-neoplastic lesions of female virgin Han:NMRI mice, incidence and influence of food restriction throughout life span. II: Respiratory tract. Laboratory Animals 19: 224-235
ADDITIONAL COMMENTS:	This lesion was first reported in aged mice, but has recently been seen in mice as young as 10-11 weeks of age. The pathogenesis is unknown, but the appearance of the lesion is suggestive of an organizing thrombus. Literature describes the lesion as being located within the tunica media; the lesion in the current case appears to lie on top of the tunica media. Special stains to identify the internal and external elastic laminae of the affected vessel should allow definitive localization of the lesion.

Slide Seminar Abstracts

Case 4: Arterial mineralization in the wall of the aortic arch in the dog

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ABSTRACT:	Arterial mineralization in the aortic arch has recently been observed in our laboratory in three male beagle dogs aged 8-8,5 months. This rather uncommon finding will be discussed in this case presentation.
ANIMAL(S):	
Species, breed	Beagle dogs from Harlan Winkelmann GmbH, Germany
Sex	Male
Age	8 to 8,5 months
Organ(s)	Aortic arch
Gross finding(s)	Except for a single sore in one male no macroscopic changes were reported in the three male animals.
Staining	H&E
CONTRIBUTOR'S MORPHOLOGIC DIAGNOSIS:	Focal slight to moderate mineralization in the tunica media of the aortic arch.
CONTRIBUTOR'S DESCRIPTION AND COMMENTS:	This change was an incidental finding observed in three male beagle dogs.
LITERATURE:	Schwarz T, Sullivan M, Störk CK, Willis R, Harley R, Mellor DJ. Aortic and cardiac mineralization in the dog. Vet Radiol Ultrasound. 2002 Sep-Oct;43(5):419-27. Kelly DF. Classification of Naturally Occuring Arterial Disease in the Dog. Tox Path. 1989; Vol. 17, Number 1 (part 2):77-93.

Slide Seminar Abstracts

Case 5: Histological evidence of epitheliotropic lymphoproliferative disease in a zebrafish

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ABSTRACT:	Fish are increasingly used as test systems in toxicologic research. Zebrafish is currently among the most popular laboratory species, yet compared to more traditional laboratory species, relatively little information on background pathology is publicly available. We observed a lymphoproliferative disorder with epidermal invasion in a single adult zebrafish (Danio rerio) which had been exposed during 4 weeks to an intermediate dose of tetrabromobisphenol A (TBBPA, a brominated flame retardant). There was marked proliferation of uniform round cells in the thymus region, extending into surrounding gills and musculature; distant intraepithelial foci were observed in the frontal cranial epidermis. Although variable cell size and pleomorphism along with an increased mitotic rate may be more typical of (piscine) lymphoma, the invasive nature of the lesion in the absence of hallmarks of tissue damage or skin ulceration indicates that the present lesion is of neoplastic nature. Epitheliotropism could be consistent with the typically relatively well-differentiated appearance of epitheliotropic neoplastic T-cells in other vertebrates and further supports a thymic origin. Apart from the cranial skin, no distant tissues were involved.
ANIMAL(S):	
Species, breed	Zebrafish (Danio rerio)
Sex	female
Age	Adult, approximately 9 months
Study type	Partial life cycle: adult 4 weeks exposure, semistatic, water borne
Treatment	Tetrabromobisphenol A, TBBPA
Clinical findings	None related to this lesion, reduced egg production in high dose
Organ(s)	Head region: Thymus, gills, cranial epidermis
Gross finding(s)	No remarkable gross findings
Staining	H&E
CONTRIBUTOR'S MORPHOLOGIC DIAGNOSIS:	Zebrafish, head region: multicentric epitheliotropic lymphoma

Slide Seminar Abstracts

CONTRIBUTOR'S DESCRIPTION AND COMMENTS:	The lesion was characterized by marked thymus hyperplasia, which extended along the primary gill lamellae. Dense aggregates of round cells invaded the neighboring branchial epithelium and more dorsally into the adjacent musculature and adipose tissue. Proliferating cells were uniform with a limited amount of distinctly bordered, basophilic cytoplasm, and central round to occasionally indented nuclei of equal sizes, with densely clumped chromatin and indistinct nucleoli. Mitotic figures were rare. A few similar cells were also present within the adjacent inner ear. Aggregates of proliferating cells were multifocally observed invading between epithelial cells of the dorsal cranial epidermis, and intracellularly into club cells (emperipolesis).
LITERATURE:	Kieser D., Kent M.L., Groff J.M., McLean W.E., Bagshaw J., 1991. An epizootic of an epitheliotropic lymphoblastic lymphoma in coho salmon Oncorhynchus kisutch. Dis. Aquat. Org. 11, 1-8. Okihiros M.S., Hinton D.E., 1989. Lymphoma in the Japanese medaka Oryzias latipes. Dis. Aquat. Org. 7, 79-87. Battalora, M.St.J., Hawkins W.E., Walker W.W, Overstreet, R.M., 1990. Occurrence of thymic lymphomas in carcinogenesis bioassay specimens of the Japanese medaka (Oruzias latipes). Cancer research (suppl.) 50, 5675s-5678s
ADDITIONAL COMMENTS:	Lymphomas have been reported in a number of fish species including medaka, salmon and trout, turbot, pike and pike-perch, channel catfish, and cod; involvement of the thymus has been observed in Japanese medaka (Oryzias latipes, another popular laboratory species), and salmonids. Thymus involvement has been observed in medaka and coho salmon epizootic lymphoma, indicative of a contagious cause. A retroviral origin was indicated for transmissible cutaneous lymphomas in pike. The present lesion was found in only 1 out of 54 adult zebrafish from this study (6 fish per tank), and similar lesions were not observed in occasionally sectioned broodstock fish or fish used in other similar studies. We therefore regard this observation as an incidental lesion; as a result of the low tumor rate, we cannot comment on a possible relation to TBBPA exposure, however the lesion was never seen in a repeated or pilot study and TBBPA is not a suspected carcinogen. The present observation indicates that epitheliotropism can occur in zebrafish thymic lymphoma. Further characterization of incidental zebrafish lymphoproliferative diseases will benefit from development of immunological markers for lymphocyte subsets.

Slide Seminar Abstracts

Case 6: A case of pseudo-placentational endometrial hyperplasia in a Beagle dog

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ABSTRACT:	When evaluating the female reproductive system in the context of preclinical toxicity studies, the pathologist needs to rely on a detailed knowledge of spontaneous background lesions and of estrous cycle-related morphological features (modifications, hallmarks) in order to adequately identify and characterize treatment-related changes. Here we present and discuss a case of spontaneous focal proliferative lesion of the endometrial mucosa in a young adult (approximately 10 months of age) female Beagle dog used in a toxicity study. The animal was part of a preliminary 7-day oral toxicity study. At necropsy focal proliferative changes of the endometrial mucosa were observed in both uterine horns. These were characterized by the presence of a yellowish or pale tan soft spongy tissue bulging on the mucosal surface of the endometrium. Histological evaluation of the mucosal changes revealed the presence of long slender folds of endometrial mucosa with a single layer of cuboidal to tall columnar epithelial cells lining a scant fibrovascular stroma. Epithelial cells were characterized by basally located abundant pale foamy-vacuolated cytoplasm, regular round-oval nuclei with vesicular chromatin located in the upper portion of the cell, and a more dense eosinophilic to amphophilic apical portion of cytoplasm. In most of the endometrial mucosal folds the apical portion abutting the uterine lumen was characterized by degeneration and necrosis of the epithelium and of the supporting stroma with sloughing of degenerated epithelial cells and debris in the lumen. In addition, accumulation of slight to moderate amounts of grey to pale basophilic mucinous material was detectable in the uterine lumen among the slender endometrial mucosal folds. The aforementioned segmental proliferative endometrial changes occurred in a morphological background of the adjacent uterine mucosa consistent with late diestrus, also confirmed by the presence of corpora lutea in both ovaries.
The overall appearance of the focal endometrial proliferative changes observed in this animal is consistent with a condition that needs to be distinguished from the classical cystic endometrial hyperplasia. Similar changes have been previously diagnosed with a spectrum of morphological designations such as “deciduoma”, “maternal placenta-like endometrial hyperplasia”, and “endometrial hyperplasia in pseudocyesis”. The most recently proposed criteria for classification of endometrial proliferative changes in the canine species would perspect a diagnosis of “pseudo-placentational endometrial hyperplasia”.
ANIMAL(S):	
Species, breed	Dog, Beagle
Sex	Female
Age	Young adult (approximately 10 months)
Study type	Preliminary 7-day oral toxicity
Treatment	vehicle control
Clinical findings	None

Slide Seminar Abstracts

Organ(s)	Uterus
Gross finding(s)	Bilateral segmental proliferative changes of the endometrium characterized by the presence of yellowish or pale tan soft spongy tissue bulging on the mucosal surface
Staining	Hematoxylin & Eosin Alcian Blue – Periodic Acid Schiff
CONTRIBUTOR'S MORPHOLOGIC DIAGNOSIS:	Uterus: pseudo-placentational endometrial hyperplasia
CONTRIBUTOR'S DESCRIPTION AND COMMENTS:	see Abstract Long slender folds of endometrial mucosa with scant fibrovascular stroma lined by a single layer of cuboidal to tall columnar epithelial cells showing basally located abundant pale foamy-vacuolated cytoplasm, regular round-oval nuclei with vesicular chromatin located in the upper portion, and a more dense eosinophilic to amphophilic apical portion of cytoplasm. The apical portion of mucosal folds shows degeneration and necrosis of the epithelium and of the supporting stroma with sloughing of degenerated epithelial cells and debris in the uterine lumen. Accumulation of grey to pale basophilic mucinous material is detectable in the uterine lumen among the slender endometrial mucosal folds. Comment: the present case further highlights the importance for toxicological pathologists to be aware of the possible occurrence of uncommon spontaneous background changes in tissues of the female reproductive system.
LITERATURE:	Hoane J, Rebelatto MC, Kiupel M (2008). Maternal placenta-like endometrial hyperplasia in three beagle dogs. Vet Pathol 45: 790 Koguchi A, Nomura K, Fujiwara T, Kawai Y, Okaniwa A (1995). Maternal placenta-like endometrial hyperplasia in a beagle dog (canine deciduoma). Exp An 44: 251-253 McEntee K (1990). Reproductive pathology of domestic mammals. Academic Press Nomura K (1997). Induction of canine deciduoma in some reproductive stages with the different conditions of corpora lutea. J Vet Med Sci 59: 185-190 Rehm S., Stanislaus D.J.,Williams A.M. (2007). Estrous cycle-dependent histology and review of sex steroid receptor expression in dog reproductive tissues and mammary gland and associated hormone levels. Birth Def Res (Part B) 80: 233-245 Schlafer DH, Gifford AT (2008). Cystic endometrial hyperplasia, pseudo-placentational endometrial hyperplasia, and other cystic conditions of the canine and feline uterus. Theriogenology 70: 349-358 Yuan YD, Foley GL (2002). Female Reproductive System, in: Handbook of Toxicologic Pathology, Haschek W.M., Rousseaux C.G. and Wallig M.A. Eds., Vol. 2, 2nd edition, Academic Press

Slide Seminar Abstracts

Case 7: Laser microdissection and quantitative real time PCR applied in characterization of IGF-1- and insulin receptor mRNA expression in the rat mammary gland

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[4]Molecular Microbiology, Intercell AG, Austria.

ABSTRACT:	Supra-pharmacological doses of the insulin analogue B10Asp, which has increased affinity for the IGF-1 receptor (IGF-1R) compared to native insulin, have been reported to increase the incidence of mammary tumors in female Sprague Dawley rats (Dideriksen et al., 1992). However, the expression of IGF-1R and insulin receptors (IR) in the rat mammary gland has not been fully examined, and the mechanisms behind the possible carcinogenic effect of B10Asp in the rat mammary gland remain unknown. The mammary gland epithelium is supported by connective tissue and embedded in the mammary fat pad (reviewed by Masso-Welch et al., 2000). Proliferation of mammary epithelium is controlled by numerous hormones and growth factors and IGF-1 is involved in paracrine signalling between the epithelial and stromal compartments (Ruan et al., 1995, Walden et al., 1998). The aim of this study was to quantify the amounts of mRNA encoding IGF-1R and IR in different compartments of the rat mammary gland relative to endogenous reference genes and compare with reference tissues known to express the receptors. P.A.L.M.´s Laser Microbeam Microdissection and Pressure Catapulting (LMPC) system was used to isolate alveolar lobules and connective tissue from cryosections of the abdominal and inguinal mammary glands collected from young, virgin, female Sprague Dawley rats. Cryosections were mounted on a PEN-membrane slide, fixed in ethanol and stained with hematoxylin and eosin using a shortened procedure, to identify the relevant mammary gland structures. The cryosections were dehydrated and immediately thereafter laser microdissection was performed for 50 minutes. For each animal, this procedure was repeated five to seven times to collect sufficient material for RNA extraction from alveolar lobules and connective tissue, respectively. RNA quality was investigated using an Agilent 2100 Bioanalyzer. RNA from microdissected mammary gland compartments was of lower quality than RNA extracted from macrodissected mammary gland. However, RNA from micro- and macrodissected tissue was all of sufficient quality for cDNA synthesis and quantitative real-time PCR. The expression of IGF-1R and IR was measured relative to a panel of ten selected reference genes and compared to liver and skeletal muscle. We found an increased expression of IGF-1R mRNA in mammary gland alveolar lobules and in the mammary gland connective tissue, compared to liver and skeletal muscle. The mammary gland alveolar lobules had a higher expression of IR mRNA than mammary gland connective tissue, but compared to liver and muscle the expression of IR mRNA was low in both mammary gland compartments. In conclusion, we have shown that it is possible to use LMPC to isolate compartments of interest from a complex tissue and extract good-quality RNA. However, laser microdissection was time consuming. Furthermore, we have added information to the characterisation of an important animal model used in preclinical safety studies, by showing that the mammary gland from young female virgin Sprague Dawley rats has a high expression of IGF-1R mRNA compared to liver and skeletal muscle, whereas the expression of IR mRNA is low compared to liver and muscle.

Slide Seminar Abstracts

ANIMAL(S):	
Species, breed	Sprague Dawley Outbred Rats (NTac:SD) from Taconic Europe, Denmark
Sex	Female
Age	10 weeks
Study type	Basic research study
Treatment	No treatment
Clinical findings	No clinical findings
Organ(s)	Mammary gland, liver and skeletal muscle (m. soleus)
Gross finding(s)	No gross findings
Staining	Hematoxylin and eosin
CONTRIBUTOR'S MORPHOLOGIC DIAGNOSIS:	N/A See abstract
CONTRIBUTOR'S DESCRIPTION AND COMMENTS:	See abstract
LITERATURE:	Dideriksen, LH, Jorgensen, LN, Dreijer, K. Carcinogenic effect on female rats after 12 months administration of insulin analogue b10 asp. Diabetes 1992; 41 (suppl. 1): Abstract 143A. Masso-Welch PA, Darcy KM, Stangle-Castor NC, Ip MM. A developmental atlas of rat mammary gland histology. J Mammary Gland Biol Neoplasia 2000;5:165-185. Ruan W, Catanese V, Wieczorek R, Feldman M, Kleinberg DL. Estradiol enhances the stimulatory effect of insulin-like growth factor (IGF-1) on mammary development and growth hormone-induced IGF-1 messenger ribonucleic acid. Endocrinology 1995;136:1296-1302. Walden PD, Ruan W, Feldman M, Kleinberg DL. Evidence that the mammary fat pad mediates the action of growth hormone in mammary gland development. Endocrinology 1998;139:659-662.

Slide Seminar Abstracts

Case 8: Unexpected Nasal Changes after Oral Gavage in Rats and the Relation to Delayed Gastric Emptying

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ABSTRACT:	While the nasal cavity is generally the first site to be affected in inhalation toxicity studies, compound-related nasal effects after oral administration are much less frequent and histology of the nasal cavity is therefore not required for subchronic oral toxicity studies. When nasal changes are observed in oral gavage studies, they are either attributed to systemic exposure to the test compound or technical gavage accidents and other causative factors are not explored. It appears however, that for certain compounds and/or compound formulations, additional factors might be important, resulting in an increased risk of gavage-related respiratory effects and mortality. Only very limited information had been published yet on this (Conybeare and Leslie 1988, De Jonghe et al. 2008). In a 3-week oral gavage toxicity study in rats with a compound in early drug development, unexpected respiratory symptoms and high mortality were noted in the compound treated groups only. At histology, the most striking lesions were extensive inflammatory and necrotic changes within the nasal cavity and nasopharynx in both preterminal and terminal rats. Characteristically, the changes were most pronounced in the posterior region of the nasal cavity and especially the nasopharynx with food particles present within the inflammatory exudate. Based on these findings, reflux of stomach content (with test article formulation) and subsequent aspiration was suspected, which may have then resulted in irritation of the upper respiratory tract. After intravenous administration of the compound no respiratory lesions or mortality were observed, suggesting a critical role of the gavage dosing method. For further evaluation, a mechanistic 2-week gavage rat study was performed comparing modified gavage procedures (e.g. fasting before dosing, reducing the dose volume by increasing the concentration or b.i.d. dosing instead of once daily dosing). In this study, lowering the dose volume from 10 to 5 ml/kg (by doubling the concentration) and fasting the rats 4 hours before dosing substantially reduced the respiratory effects and mortality. Surprisingly, reducing the concentration only and changing to b.i.d. dosing had no beneficial effect. Based on these observations, the volume of stomach contents at the time of the gavage seemed to play a crucial role. Since respiratory effects were seen in treated rats only, a compound-induced effect on gastric emptying was suspected as an explanation as to why reflux-related nasal lesions were seen in treated animals only. This was confirmed in an additional pharmacology study, which revealed a pronounced dose dependent enlargement of the stomach with increased content, indicative of delayed gastric emptying. Conclusion: Delayed gastric emptying in rats may be a critical factor in oral gavage studies, accounting for unexpected respiratory effects and mortality due to gastro-esophageal reflux and subsequent aspiration of irritant material upon removing the gavage tube. It is important to differentiate this phenonomenon from technical gavage accidents since reflux may be mitigated or eliminated by reducing the dose volume and dosing in a fasted state. Furthermore, the presented data show, that histological examination of the nasal cavity may reveal important evidence for gastro-esophageal reflux and helps to differentiate from compound-related toxicity.
ANIMAL(S):	RAT
Species, breed	Sprague Dawley

Slide Seminar Abstracts

Sex	male
Age	approx. 6 weeks at start of the study
Study type	Mechanistic 2-week oral gavage toxicity study
Treatment	Compound X in early development
Clinical findings	Dyspnea, audible respiration, excessive salivation and reflux on day 7 and 8 of dosing; preterminal euthanasia on day 8 due to moribund condition
Organ(s)	Nasal Cavity (level II and level III) and Nasopharynx (4 levels)
Gross finding(s)	-
Staining	H&E
CONTRIBUTOR'S MORPHOLOGIC DIAGNOSIS:	Nasal cavity: extensive ulceration/necrosis with acute inflammation affecting mucosal surfaces (without targeting a specific cell type or tissue structure) and extending into deeper tissue layers up to the bone; multiple adhesions of nasal turbinates/septum.
CONTRIBUTOR'S DESCRIPTION AND COMMENTS:	Unspecific and diffuse alteration pattern: extensive necrosis and inflammation within the nasal cavity without targeting a specific structure or cell type - indicative of direct irritation due to inhalation of irritant material rather than due to systemic exposure related toxicity. Specific distribution pattern: most pronounced lesions in posterior nose levels and especially involving the nasopharynx with occasional presence of food material within the inflammatory exudate – suggesting retrograde aspiration of stomach content and thus indicative of gastro-esophageal reflux.
LITERATURE:	- Conybeare G and Leslie GB (1988): Improved oral dosing technique for rats. J Pharmacol Methods 19(2): 109-16. - De Jonghe S. et al. (2008): Lethal rhinitis/sinusitis in rodents by aspiration of formulation in gavage studies: importance of evaluation of the nose. 6th European Congress of Toxicologic Pathology, Edinburgh, Poster

Poster Abstracts

P1: Reactive vascular proliferative changes in the mesenteric lymph nodes of aging CD 1 mice

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The mesenteric lymph node of aging mice is the most susceptible site for the development of hemangioproliferative changes according to historical incidences in the RITA database. In own carcinogenicity studies, in CD 1 mice, high incidences of reactive angiomatous changes were found in the mesenteric lymph node. They were characterized by incipient proliferation of blood vessels including vascular transformation of sinuses and formation of new small vessels in the paracortical area leading to atrophy of the lymphoid tissue. Hemangiectasia, erythrophagocytosis, pigment deposits, formation of new trabecula and prominent hemopoiesis were frequently seen in affected lymph nodes whereas fibrosis was insignificant. Abundant erythrocytes in the lymph node sinuses suggested an anastomosis between blood vessels and lymph sinuses.

This type of change is known in humans as sequel of obturation of effluent veins or lymph vessels and could be experimentally reproduced in rabbits by Steinmann et al. (1982). In mice, it is often not possible to determine the immediate cause of the lymph node changes in routine sections taking into consideration the complex draining situation of the mesenteric lymph node. However, a variety of changes typical for aging mice may come into account as for example marked enlargement/distension of female reproductive organs, liver tumor, systemic amyloidosis, or thrombosis.

In the lymph node, it is often challenging to distinguish the reactive type of changes from angiomatous hyperplasia or hemangioma since vascular channels with a monolayer of endothelial cells are typically found in all of these lesions. The following features suggest a reactive lesion: vascular channels lined by a normal-appearing endothelium, localization within the boundaries of the lymph node capsule, focal ectasia of lymph node sinuses or veins, and hemopoiesis.

Considering the relatively high incidence of angiomatous hyperplasia and hemangioma and the common observation of reactive vascular changes specifically in the mesenteric lymph node of mice, a relationship between the development of vascular neoplasias and disturbed circulation of blood or lymph has to be taken into account.

Literature

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Poster Abstracts

P2: Lead nitrate exposure induces structural changes but not the DNA fragmentation in the rat liver

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A ubiquitous environmental toxicant- lead is known to affect several organ systems. This study was designed to investigate the effects of lead nitrate exposure on liver structure and DNA fragmentation. Lead nitrate was orally exposed at the dose-levels of 0, 0.5% and 1% for 60 days and sacrificed on next day. The liver was processed for thick sections evaluated by toluidine blue staining, and electron microscopy after staining with uranyl acetate and lead citrate. The DNA damage was assessed by DNA fragmentation assay. There was no effect on weight of livers in lead treated groups (P>0.05). Lead nitrate qualitatively affected the structure in terms of necrosis, induction of small and large vacuoles, dilatation of sinusoids, decrease in brush border, displacement of organelles, encroachment of nuclei by the vacuoles and occasional leukocyte infiltration. The qualitative changes were in a dose-dependent manner. Ultrastructurally, hepatocytes, Kupffer cells or Ito cells did not present any characteristics of apoptosis, and the nuclear diameter was also not altered by lead (P>0.05). The DNA fragmentation assay revealed no significant changes in DNA damage (P>0.05) further supporting the fact that the lead exposure did not induce apoptosis. In conclusion, lead nitrate induces subtle necrotic changes and formation of vacuoles, which eventually result in liver damage.

Poster Abstracts

P3: Identification of new structural alerts for hepatotoxicity by cluster analysis of a human adverse effects data set

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Drug attrition due to adverse effects in the liver is a recognised issue for the pharmaceutical industry. In silico methods that can predict the potential hepatotoxicity of a compound based on its chemical structure provide a valuable tool in helping to screen out such compounds during the initial stages of drug development. A knowledge-based expert system approach has previously been used to develop 40 structural alerts, based on published human and animal toxicity data, for the prediction of hepatotoxicity. These describe structure-activity relationships for the hepatotoxicity of a variety of chemical classes. In efforts to further expand the coverage of alerts, a number of approaches are being investigated. One of these is the use of compound clustering techniques to identify candidate classes for development as new structural alerts. The application of this method to a data set of pharmaceuticals and their associated adverse effects is described here.

A data set of 1609 compounds, with post-market report activity scores for five liver toxicity endpoints (liver damage, jaundice and cholestasis, liver enzymes, gall bladder disorders and bile duct disorders), was investigated. The current performance of the existing liver toxicity alerts against the data set was evaluated. The data set was then analysed using software tools to generate clusters based on the similarity of chemical features. The generated clusters were assessed for their suitability as the basis of potential structural alerts, according to the criteria that each valid cluster must contain a minimum of four compounds and that more than 40% of these must be considered active for at least one of the five endpoints. The shortlisted clusters were visually analysed together with their toxicity data to identify the final candidate classes.

Initial processing of the dataset against existing hepatotoxicity alerts gave sensitivity scores of 29 to 36% and specificity scores of 76 to 78% for the hepatotoxicity predictions across the five endpoints. A total of 213 clusters were generated, out of which 18 satisfied the selection criteria described. From these 18 classes, five are currently included amongst existing hepatotoxicity alerts. Seven more clusters were found to overlap with alerts currently in development which have been generated using other methodologies. The remaining six classes were visually analysed and five were identified as candidates for potential alert development. Examples include the papaverine and substituted uracil/cytosine classes.

The results of this work have demonstrated that candidate hepatotoxicity structural alerts can be discovered using a software clustering approach. The candidate structural alerts derived here differ from alerts developed previously in that they make use solely of post-marketing adverse effect report data and hence have particular relevance to the prediction of hepatotoxicity in humans. Further investigation of the published literature will potentially provide additional toxicity data and mechanistic insight in support of these structural alerts.

Poster Abstracts

P4: Hepatoprotective effect of Curcuma longa L. in D-galactosamine induced liver injury in mice: Evidence of antioxidant activity

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The aim of this study was to investigate the hepatoprotective effect of methanolic extract of *Curcuma longa* L (CLME) in D-galactosamine (GNH2) induced liver injury and the mechanism(s) involved. The ability of vitamin C (VC) to attenuate the toxicity was also examined.
Mice were orally pretreated with CLME and VC at a dose of 100-mg/kg for seven consecutive days before challenge with a dose of GNH2 (800 mg/ kg i.p.). Integrity of liver from the animals was assessed by determining the levels of serum alanine and aspartate aminotransferases (ALT and AST) and alkaline phosphatase (ALP). The antioxidant status was monitored by the levels of hepatic superoxide dismutase (SOD), catalase (CAT), glutathione-s-transferase (GST), glutathione (GSH) and malondialdehyde (MDA) (Lipid peroxidation (LPO) index). GNH2 treatment markedly increased the levels of serum ALT and AST, which were significantly ($p<0.05$) attenuated in animals pretreated with CLME and VC. Also, CLME significantly ($p<0.05$) increased the levels of hepatic GST and SOD, with a concomitant marked reduction in the levels of hepatic and serum LPO in the GNH2-challenged mice. Furthermore, hepatic GSH which was decreased after GNH2 intoxication, was significantly ($p<0.05$) enhanced by co-treatment with CLME and VC. However, there were no significant differences ($p>0.05$) in the levels of ALP and CAT of these animals. The liver histopathology results revealed that GNH2-induced injury was prevented in mice co-treated with VC and CLME. The results suggest that the hepatoprotective effect of CLME in GNH2 induced liver injury may be related to its antioxidant activity.

Poster Abstracts

P5: Effects in CD-1 mice liver following prenatal DEHP exposure

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Needs Improvement Bis(2-ethylhexyl)phthalate (‚DEHP) is widely used as a plasticizer in manufacturing of articles made of PVC, as baby-toys, medical devices (trasfusio/infusion sac, catheters etc) and home tools as well. Plastics may contain 1% to 40% of DEHP. Recent studies showed that population is mainly exposed to DEHP trough food and consumer products, since it not covalently bound to PVC and can be released into foods, environement and biological fluids. Biomonitoring studies detected DEHP in different human matrices such as umbilical cord blood and mother's milk. Pre-term neonates, who are treated by medical devices such as gastric, respiratory probes or catheters, represent a group at high risk of DEHP exposure.

DEHP is considered an Endocrine Distrupter (ED) because it interferes with androgen metabolism impairing male reproductive development in rodents. In adult rodents DEHP induces hepatocarcinogenesis through peroxisomes stimulation by the Peroxisome Proliferator-Activate Receptors (PPARs), a nuclear transcriptional receptors' family which modulate gene expression of glucose-carrier and lipids-carrier proteins. PPARα and PPARγ arer higly expressed in liver and adipose tissue, respectively. The induction of PPARα by DEHP showed in rodents, is not yet proved in humans.

Whereas DEHP – induced chronic hepatic effects are well investigated in adult rodents, little data exist on the possible correlation between DEHP exposure in susceptible developmental phases with metabolic and cancer-biomarkers alterations in liver.

CD-1 pregnant mice (n= 10/group) were treated by oral admistration with 0, 25, 100 mg/kg bw day of DEHP on gestational days 11 -19 (corresponding to liver organogenesis and histogenesis in mice). General health conditions, body weight gain and food consumption were daily checked and recorded. Dams delivered spontaneously. Pups were weighted, counted and checked to detect possible malformations or premature death on postnatal day (PND) 1 and every 4 days. At PND21 (mouse weaning) the dams and half of the pups of each litter were subjected to blood sampling from retro-orbital plexus prior anesthesia and then suppressed by CO2 asphyxia. At PND35 (mouse puberty) the remeaning pups were sacrificed. Livers were excised from every animal, weighted and fixed in 10% buffered formalin for subsequent histological analysis (haematoxilin-eosin -E&E- and periodic acid schiff –PAS) as well as Immunohistochemistry (IHC) to determine ß-catenin localization..

Both DEHP-treated groups showed lower weight gain of dams during pregnancy as well as lower litter size and litter weight at birth., At PND21 litter weight was lower at 100mg/kg. No effect on liver weight was observed in either dams or pups. Liver histological examination showed.in both treated groups at PND21 dose releated increases of hepatocyte vacuolation (suggestive of fatty change), pyknotic nuclei, leukocytes imphiltration and hematopoietic foci. This pattern of histological alteration waas essentially unchanged at PND35. Effects were significantly more evident in male pups suggesting a gender-related DEHP effect in liver. PAS staining showed a marked reduction of hepatocyte glycogen storage, present with dose-related severity at both dose levels. The glycogen reduction was essentially the same at both sampling times and in either sex. IHC showed a dose-related and sex-related increase of cytoplasmic localization of ß-catenin; the increase was observed in females of both treated groups at PND21 and of top-dose group at PND35, whereas it was present in top-dose males at PND21 only.

Our data indicate that prenatal exposure to DEHP can induce complex, sex-related patterns of effects on liver histology and metabolic programming.

Poster Abstracts

P6: Air pollution, heart inflammatory signaling molecules and chocolate

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BACKGROUND: Air pollution is a serious environmental problem. Elderly subjects have a high risk for cardiac and respiratory diseases. Mexico City residents are exposed to high concentrations of air pollutants including $PM_{2.5}$ and endotoxins, and exhibit respiratory and systemic inflammation and cardiac autonomic dysfunction.

OBJECTIVES: To test the hypothesis that chronic exposure to severe urban pollution produces myocardial inflammation in Balb-c mice exposed naturally to two distinctly different polluted areas within Mexico City (MC): SW high in endotoxins, and ozone and NW an industrial area with heavy traffic, higher PM_{10} and $PM_{2.5}$ and PM associated metals.

METHODS: Four groups (n:10-12 each) of Balb-c female mice 16m old from clean air, NWMC, SWMC, and SWMC mice chronically treated with oral dark chocolate containing 47.6 mg of polyphenols. RT-PCR for IL-1β, CD14 and COX2 mRNA expression in myocardium.

RESULTS: There was a significant upregulation of inflammatory genes in the Mexico City exposed mice compared to controls, including IL6, TNF alpha, IL1 beta and the LPS receptor CD14.IL-1β was significantly higher in the SWMC group versus the CTL and NWMC mice (p=0.0012) and chocolate significantly decreased its expression (p=0.02).TNF α was significantly reduced in the chocolate treated mice p<0.0001. IL6 was higher in NW mice and decreased expression in the chocolate treated SW animals was significant at p=0.01.CD14 was higher in the SWMC group in comparison to the CTL group (p=0.0003.

CONCLUSIONS: 16 month-old Balb-c mice with life long exposures to the polluted Mexico City atmosphere (compared to clean-air controls) exhibited significant myocardial inflammation.

Southwest Mexico City mice had the highest CD14 myocardial expression in keeping with the highest endotoxin PM 10 levels in the area.

<u>The oral chronic (15 months)administration of dark chocolate-rich in flavonoids to SWMC mice significantly reduced their myocardial inflammation.</u>
Southwest Mexico City elderly residents could benefit from the chronic intake of dark chocolate for maintaining cardiovascular health.

Poster Abstracts

P7: Bleomycin-induced lung fibrosis model in the mouse

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The bleomycin-induced lung fibrosis model in rodents is used to evaluate the pathophysiology of fibrotic lung diseases. A single intratracheal instillation of bleomycin induces lung injury from bleomycin-mediated cleavage of DNA. At first, it induces an inflammatory reaction, which lasts for about 9 days. This inflammatory response causes damage to the airway epithelium, activation of fibroblasts and subsequent pulmonary fibrosis.

Histologically, intratracheal instillation of a single dose bleomycin results mainly in patchy parenchymal inflammation of variable intensity, epithelial cell injury with reactive hyperplasia, basement membrane damage, and a bronchiolocentric (to coalescing) distribution of interstitial and intra-alveolar fibrosis.

This mousse model can be used to distinguish anti-inflammatory and anti-fibrotic actions of test compounds. Therefore, it is speculated that comparison of pharmacologic treatment starting at day 0 (preventive treatment) with treatment starting at day 10 (therapeutic treatment) would allow us to distinguish anti-inflammatory and anti-fibrotic actions of test drugs. Compounds inhibiting the initial inflammation should prove active when administered over the entire first period and ineffective if administered after the bulk of the inflammatory response has resolved (after day 10). In contrast, anti-fibrotic agents should prove effective irrespective of the treatment mode.

The above described model will be illustrated by a Single Dose Intratracheal Toxicity Study of Bleomycin in the C57BL/6J Rj mouse, used to determine the optimal dose of bleomycin for the induction of pulmonary inflammation and fibrosis.

Poster Abstracts

P8: Index values in the differential bone marrow count in Sprague-Dawley rats: available tools to evaluate haematotoxicity

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Alteration of the haemopoietic system is a common dose-limiting factor of drugs in preclinical and clinical studies. Assessment of the bone marrow smear has a significant relevance for different therapeutics, particularly for cancer and growth factor drugs.

Application of reference values and indices to the bone marrow smear is a useful tool to compare different test items that can improve cytopenia (cancer drugs) or stimulation of hematopoiesis (growth factors).

This study provides a practical example of two different test items with a similar pharmacological activity. The calculation of bone marrow indices was applied to both sexes of eight-week old Sprague-Dawley rats. A complete examination of bone marrow smears was performed for animals receiving the test and reference item in order to establish the bone marrow indices. In particular, the M:E index (Myeloid/Erythroid ratio) and subsequently the subdivision of cells in maturative and proliferative pool, EMI (Erythroid Maturation Index), I:Me (Erythropoiesis Index), I:M (Myelopoiesis Index), I:Mg (Granulopoiesis Index), MMI (Myeloid Maturation Index) were calculated.

The reference values, for differential counts of myeloid and erythroid cell lines and related indices, give useful information, for each cell line compartment, to characterise any maturation abnormality and provide the basis for an appropriate interpretation and evaluation of the test item, when compared with the reference item.

The above-mentioned bone marrow analysis is an important component for toxicity or safety assessment studies.

Poster Abstracts

P9: Toxicity effect of vincristine –treatment on mice testis tissue

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Introduction: Vincristine is an Alkaloid derived from Vinca-rosea that has its mode of action in an inhibition of mitotic processes due to binding to the protein tubulin in mitotic spindles, with formation of cytoplasmic inclusion bodies. Its major use is as an antineoplastic against acute leukemia's and certain lymphomas and neuroblastomas. Vincristine is known to have testicular alterations as a side effect. Therefore, we have tried in this study to evaluate the toxic effects of this drug on Mice testis tissue.

Materials and Methods: In this study we choose 40 adult male mice (please indicate strain). The mice were divided randomly into 2 groups as Control and Experimental. Mice of the experimental group were 3 times injected intraperitoneally with Vincristine (3 mg/kg/week).The Control group was injected by normal Salina. At the termination of the experiment the testes were fixed (please indicate fixative) and stained with H&E and Trichrome Masson. Data were analyzed by ANOVA and Tukey test and SPSS software.

Results: The microscopical examination revealed the following changes: The diameter of seminiferous tubules and epithelial thickness and the average percentage of tubules with spermatozoa was significant diminished. The spermatogenic cells were significantly decreased showing degenerative changes. In comparing the average of the parameters in the experimental group with control group, there was significant difference (P<0.05).

Conclusion: This study suggests that Vincristine impairs spermatogenesis and testis function. These findings will have an important bearing for the treatment of young patients.

Poster Abstracts

P10: Predicting rat carcinogenicity findings using chronic study results

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The PhRMA PSLC Carcinogenicity Working Group, a collaborative initiative involving 13 companies, was launched with the goal of improving speed and optimizing resource commitment to assess pharmaceutical carcinogenicity risk. Histopathology findings of foci of cellular alteration, hypertrophy, hyperplasia and neoplasia in 6- and 12-month rat toxicity studies were compared to carcinogenicity findings in 2-year rat bioassays for 182 compounds to determine the ability to predict carcinogenicity outcomes based on microscopic findings from 6- or 12-month rat toxicity studies.
All available 2-year rat carcinogenicity summary data from the 13 pharmaceutical companies and the matching rat chronic (6- and/or 12-month) study data, conducted over more than 25 years, were compiled into a searchable database. Based on histopathology results and carcinogenicity outcome, compounds were classified into four categories (True Positive, True Negative, False Positive and False Negative).

Analyses of 182 compounds indicated that site-based tumor prediction was poor, but overall negative predictivity was very good on a whole animal basis.
Considering 12 m versus 6 m rat chronic study histo data did not result in a substantial improvement in the false negative Rat carcinogenicity outcome prediction.

For rat False Negative compounds, genetic toxicology results, mouse carcinogenesis study results, and evidence of endocrine hormone perturbation were evaluated as risk factors and marketing information was entered.

Integration of chronic rat study preneoplasia histo on a whole animal basis with genetox results, and evidence for chronic rat hormonal perturbation, demonstrated approximately 80% sensitivity and negative predictivity for rat carcinogenicity outcome. The majority of the false negative compounds (14/182) were single species, mostly single sex, and mostly single organ rat tumorigens of questionable human relevance.

Eliminating 2-yr rat carcinogenicity testing for compounds negative for microscopic proliferative changes in chronic studies, negative for genotoxicity and negative for hormonal perturbations would yield significant reductions in development time and animal use.

Poster Abstracts

P11: Spontaneous multiple intestinal neoplasia in B6C3F1 mice

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Although spontaneous multiple intestinal tumors have been reported in the C57BL/6JApc$^{min/+}$ (Apc^{min}) mice which bear a mutation in adenomatous polyposis coli (Apc) gene, such tumors have never been reported in any other strains of mice. This paper describes the morphological characteristics of spontaneous multiple intestinal tumors observed in the jejunum and ileum of two B6C3F1 male mice.

They were two out of 330 B6C3F1 male mice which were purchased from CLEA Japan Inc. (Shiga, Japan) and subjected to a carcinogenicity study. The animals were housed individually in wire mesh cages under controlled conditions and fed CRF-1 diet (Oriental Yeast Co., Ltd., Tokyo, Japan) and tap water ad libitum through the experimental period. The first case, from the control group, died at 41 weeks of age, showing no clinical signs, and the second case, from the treated group, was killed at 98 weeks of age under moribund state. After complete necropsy, all tissues were fixed with 10% phosphate-buffered formalin. Four-µm paraffin sections were stained with hematoxylin and eosin. In addition, immunohistochemical staining for β-catenin was also done on some selected sections of the intestine.

Macro- and microscopic characteristics of the tumors were common to both cases. Namely, serosal surface of the tumors was irregular in shape due to folding of the intestinal wall. When the intestine was incised, sessile or plaque-like nodules, 8x6x3mm to 2x2x1mm in size, were observed sporadically in the mucosal surface from the jejunum to ileum. Histologically, the masses formed papillary fronds or plaque-like lesions, and occasionally projected into the lumen showing little compression of surrounding mucosa. Neither penetration into the muscularis mucosae nor local invasion into the tumor stroma was evident. The lesions consisted of irregular-shaped glands lined by tall columnar or cuboidal epithelium, which partially included goblet cells or Paneth cell-like cells. Distortion of the gland and branching of the stroma were present, and some of the glandular lumina were filled with cell debris. Lymphocytes and plasma cells infiltrated in the stroma. The tumor cell had basophilic cytoplasm, hyperchromatic nucleus and frequently contained intracytoplasmic vacuoles. Numerous mitotic figures were detected. All masses showed same histological feature. Immunohistochemical examination for β-catenin revealed positive reaction in both the cell membrane and cytoplasm of neoplastic cells.

Consequently, the present tumors were diagnosed as multiple intestinal neoplasia of spontaneous nature and were the first cases in mice of other than APC^{min} strain.

Poster Abstracts

P12: Cardiovascular effects in rats following exposure to a receptor tyrosine kinase inhibitor

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The receptor tyrosine kinase receptor (RTK) signaling pathway, cMet, has been implicated in oncogenesis and is a target of interest in cancer therapy. PF-04254644 is a potent and selective inhibitor of c-Met. Wide ligand binding profiling of PF-04254644 revealed a potentially significant interaction with PDE3, and follow up PDE binding profile confirmed PF-04254644 is a potent inhibitor of PDE3 as well as other PDEs (1, 2, 5, 10, and 11). Clinical observations, laboratory, and echocardiography parameters were recorded in Sprague-Dawley (SD) rats that received PF-04254644 orally on 7 consecutive days of dosing. Toxicological evaluations revealed myocardial degeneration as an adverse event at dose levels equivalent to the predicted efficacious dose in humans. Echocardiographic evaluations revealed significant hemodynamic changes that correlated with the histopathologic finding of myocardial fibrosis and a sustained increased heart rate (HR) and increased contractility over 24 hrs after a single high dose. A study in telemetry instrumented rats found that both PF-04254644 and milrinone, a known PDE3 inhibitor, induced a sustained increased HR and decreased contractility. The data suggest that inhibition of multiple PDEs by an RTK inhibitor may induce cardiotoxicity in rats.

Poster Abstracts

P13: Quantifying Huntingtin protein aggregates in a transgenic mouse model of Huntington's Disease

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Background and Aims: Huntington's Disease (HD) is a progressive neurodegenerative disease that causes debilitating motor and cognitive deficits in those afflicted with the condition. The phenotypic impairments caused by HD are associated with the intracellular and extracellular aggregation of the protein, Huntingtin (HTT), in the striatum and cortex of the affected brain. These proteins are over-expressed and aggregate due to a poly-glutamine (CAG) repeat mutation in the HTT gene. HD has been well studied in recent years due in large part to the development of transgenic rodent models of the disease. Historically, analyzing neurohistological preparations from a glass slide have been difficult from a quantification standpoint due to the size of tissue sections. However, digital whole-slide imaging systems and image analysis software allow researchers to qualitatively and quantitatively evaluate entire tissue sections using a single system, dramatically enhancing the speed and accuracy of such research studies. In this report, we quantify and compare HTT protein aggregate density within the striatum and the cortex of coronal brain slices prepared from a transgenic HD mouse model, the Q140 Knock-in, using whole slide imaging and image analysis. Methods: Brain slices from Q140 animals were immunostained with an anti-HTT antibody and scanned using the Aperio ScanScope XT® instrument. Digital images were analyzed using the ImageScope® software package with the Nuclear analysis tool. Results and Conclusions: The results clearly show that HTT protein aggregation is more severe in the striatum compared to cortex for the same brain sections of the same transgenic animal. This is a significant finding, as it is clear that the physical symptoms of HD not only manifest sooner, but also more severely than the cognitive disorders associated with the disease. Future investigations using this HD transgenic mouse model should include a developmental time course of HTT protein aggregation in the brain that is correlated to motor and cognitive behavioral performance. While it will be important to segregate the differential aspects of the animals' behavioral performance (motor impairments vs. cognitive impairments) in these experiments, the ability to obtain an accurate quantitative assessment of histopathology is clearly achievable.

Poster Abstracts

P14: Evaluation of automated histology pattern recognition tool for selection and analysis of tumor regions in breast sections stained with ER, PR and Ki67

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Background and Aims: Advances in genomic and proteomic technology have generated many candidate biomarkers for tumorigenesis & cancer progression. In oncology, immunohistochemical (IHC) biomarkers are being developed as companion diagnostics to guide treatment decisions, to monitor tumor progression, and predict outcomes (reviewed by Ludwig & Weinstein, 2005). For breast cancer, a number of IHC biomarkers are analyzed routinely, including HER2, ER, PR, p53 and Ki67. Image analysis is often a bottleneck in IHC biomarker development, particularly in cancer research. Automated image analysis of tumors is complicated by the presence of both tumor and non-tumor cells (primarily stroma and infiltrating immune cells) in tissue sections. The objective of this study was to evaluate the ability of a histology pattern recognition algorithm (GenieTM) to automate the isolation of tumor tissue from IHC-stained sections for subsequent image analysis. Methods: A series of breast tissue sections stained for nuclear biomarkers, ER, PR, and Ki67, ans were scanned using an Aperio ScanScope instrument. The digital images were analyzed using a Genie classifier algorithm to select tumor tissues & a nuclear algorithm was applied to analyze stain intensity. These results were compared to selection of neoplastic regions using manual annotation. Results and Conclusions: For ER, PR and Ki67 stained slides, the percentage of positively stained cells varied by 0.9%, 0%, and 5.6%, respectively, when the two methods of tumor tissue selection were compared. For all three biomarkers, the overall intensity score was unaffected by the method of tissue selection. These results suggest that automated tumor tissue classification using histology pattern recognition may be a viable & time-saving alternative to manual tissue annotation for evaluating biomarker expression in neoplasms. These methods may be applied to facilitate the analysis of large biomarker panels, speeding the identification of the most promising biomarkers for further development.

Poster Abstracts

P15: Validation of immunohistochemical staining, concerning either markers for research use or monoclonal antibodies for therapeutic purpose, according to the Good Laboratory Practice principles at CIT

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For every new reagent used at CIT in immunohistochemistry, a stringent and comprehensive validation plan is undertaken, whether for monoclonal antibodies intended to be used as markers of toxicity or for those developed for human therapeutic use. When comprehensive, it consists of the determination of specificity, optimal concentration [C] to be used in tissues, repetitivity, reproducibility, linear area and dynamic range (from concentration without background noise to extinction of signal).

This process is slightly different when dealing with antibodies for research use only or when validating the assay for antibodies or constructs thereof intended to be used for human therapy. It is most comprehensive in tissue cross reactivity studies following Good Laboratory Practice principles.

The linear range of the concentration-response curve is of uppermost importance in the case of [C] determination for antibodies or constructs thereof intended to be used for human therapy and is best determined by image analysis. However, intra and inter-replicate variations are sometimes quite high and the consequence is sometimes to go back to a purely qualitative validation. In addition to using the best technical quality, such as standard section thickness, the use of digital slide acquisition and whole tissue section using new approaches in image analysis, such as for instance the Genie® software, could significantly improve intra- and inter-replicate variations of more classical image analysis approaches.

Poster Abstracts

P16: Next generation virtual slide based workflows for toxicological pathology

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Digital Pathology involves the creation of high resolution, diagnostic quality digital images from glass slides. Telepathology is the sharing of this image data efficiently over networks to allow remote review and diagnosis. Digital Pathology is transforming pharmaceutical pathology at both drug discovery and drug safety assessment.

Digital pathology can deliver numerous benefits to pathology in pharmaceutical companies. Pathology can be globalised through consolidation of images in a centralised servers and subsequent distribution over networks to pathologists in different international centres. This enables complete flexibility over caseload distribution and facilitates the easy sourcing of second opinion on difficult cases. Clearly this approach also allows easy interaction with Clinical Research Organisations (CROs) or alternative outsourcing approaches. Digital pathology enables the archiving of interesting cases, decision support through previously annotated materials, the establishment of a reference case portfolio and the development of high quality educational content. It offers the opportunity to consider novel approaches to existing workpractices. As material is transformed from a glass slide to a digital image, quantitative image analysis based interpretation of slides can be used both now and in the future (with greater evolution of algorithms) to automate routine aspects of pathology.

While there are considerable benefits to digitial pathology several challenges exist to enable routine use of digital pathology in the pharmaceutical industry. Digital pathology involves a complete change of current workpractices, pathologists have to move from microscope to computer monitor. Significant capital investment in new technlogies are required; slide scanners, storage infrastructures (including disaster recovery), software to enable archive management and distribution, new workstations with high definition monitors. New steps are introduced into tissue processing slide scanning along with resultant image QC and archiving. The pathologists workload is high, therefore high throughput is essential on two fronts. Firstly the required caseload has to be delivered to the pathologist on time (irrespective of scanning time) and secondly that the review of material on a computer monitor is at least of equivalent speed to the use of a microscope.

Most importantly, new next-generation workflows are required to help deliver the throughput required for routine toxicological pathology use. Firstly automatic QC of slides is critical – computer vision based approaches can be applied to help achieve this objective. Secondly appropriate Barcode/Slide Label reading for population of meta data and subsequent derivation of additional meta data from third party information systems. Thirdly, high performance caseload retrieval using powerful search engines. High performance viewer with embedded observation capturing and embedded dictionaries of pathology terminology. Easy retrieval of study data on demand along with tight integration into study management information systems.

Slidepath are working with leading pharmaceutical to help deliver these workflows to ensure that digital pathology brings real benefit to end users in the toxicological pathology space.

Poster Abstracts

P17: Intra-cardiac ectopic thyroid in a Han Wistar Rat: Case report and review of the literature

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This is the first reported case of ectopic thyroid in the heart valve of a Han Wistar rat. The rat was part of a preclinical toxicologic study and did not present with any clinical signs. At histological examination, the ectopic tissue was approximately 600 x 400 µm, located in the sub-endothelial connective tissue of the aortic valve and protruding into the left ventricular lumen. It was composed of single-layered flat to cuboidal epithelium organised in follicles often containing colloid, scattered in a loose fibrous stroma, thus ressembling mature thyroid tissue. Ectopic thyroid has been documented in many mammals. It is a relatively frequent condition occurring in various locations from the tongue to the diaphragm. The structure and function is the same as the main thyroid gland except for a complete lack of C-cells. Some cases have associated goiter or neoplastic transformation. In dogs and humans, intra-cardiac ectopic thyroid is rare and most commonly observed in the interventricular septum within the right ventricle. In rodents, no data are available about intra-cardiac ectopic thyroid and our finding is, to our knowledge, the first reported case of a heart valve position of ectopic thyroid. The occurrence of remnants of mature thyroid tissue in an ectopic location is also of interest in laboratory animals because of the possible interference with thyroid function investigation after thyroidectomy, and confounding effects of xenobiotics on thyroid tissue in shorter term or long-term carcinogenicity studies.

Poster Abstracts

P18: Protective effect of Nigella Sativa seeds extract on CCl4-induced hepatotoxicity in albino rats (toxicological, biochemical and histological studies)

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INTRODUCTION: Chronic liver diseases commonly result in liver fibrosis. Carbon tetrachloride (CCl_4) is widely used for experimental induction of liver fibrosis. CCl_4 is a potent hepatotoxin producing centrilobular necrosis which causes liver injury.

AIM: This study was carried out to investigate the role of Nigella Sativa (NS) extract on the prevention of carbon tetrachloride (CCl_4)-induced liver fibrosis in rats.

MATERIAL AND METHODS: Thirty adult male Sprague Dawley albino rats were used. They were divided into three groups: I-control group (10 rats) which was subdivided into two groups (5 each) Ia that received only tap water and Ib that received N. Sativa extract in a dose of 800 mg/kg orally every day for four weeks. II-CCl_4-induced hepatotoxicity group (10 rats). The rats in this group received CCl_4 in a dose of 0.15ml /100g body weight s.c for 3 days /week for four weeks. III-hepatotoxicity and N. Sativa extract-treated group that received CCl_4 in a dose of 0.15ml /100g body weight s.c. for 3 days /week, in addition to an extract of N. Sativa seeds at 800 mg/kg orally every day for four weeks. After 4 weeks, all rats were sacrificed. Blood samples were collected. Plasma levels of aspartate transaminase (AST), alanine transaminase (ALT), and malondialdehyde (MDA) were determined by biochemical methods. Erythrocyte superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities were also determined by biochemical methods. The liver was sampled and one portion was used to analyze the levels of nuclear factor-κB (NF-κB) by western blotting. Another sample was used for light and electron microscopic histological examination.

RESULTS: The CCl_4-induced hepatotoxicity group II showed significantly increased AST, ALT, and MDA plasma levels compared to both control groups. I divided the control into two groups to study the effect of nigella alone as it was found that the fixed oil of N. Sativa inhibit non enzymatic lipid peroxidation in liposomes. Therefore, the antioxidant action of N. sativa may be related to the preservation of intracellular glutathione (the depletion of which by oxidative stress is known to increase the susceptibility of cells to irreversible injury.so I want to compare between positive control (nigella) with antioxidant action on normal function of liver and negative control (tap water). The hepatotoxicity and N. Sativa extract-treated group showed marked inhibition of all above mentioned biochemical parameters.

Also it was found that mean erythrocyte GSH-Px and SOD levels were found to be significantly lower in the CCl_4 induced group compared with the control one while the levels of these parameters were found to be significantly increased after NS treatment. Because these two markers are indicators of oxidative stress and lipid peroxidation, so they much decreased with ccl4 but return again to nearly normal witrh treatment of rats with N. Sativa oil The expression of NF-κB in the liver of the hepatotoxicity and N. Sativa extract-treated group was lower than that of the CCl_4-induced hepatotoxicity group. Regarding histological changes, the livers of the the CCl_4-induced hepatotoxicity group showed moderate fatty degeneration and slight to moderate liver cirrhosis, whereas the livers of animals from the hepatotoxicity and N. Sativa extract-treated group showed a nearly normal architecture pattern as confirmed by light and ultra structural examination.

CONCLUSION: It is concluded that NS decreases the liver enzymes and increases the antioxidant defense system activity in the CCl_4-treated rats. N. sativa may be used in CCl_4-induced hepatotoxicity rats to prevent lipid peroxidation, increase anti-oxidant defense system activity and also prevent liver damage.

Poster Abstracts

P19: The protective effect of Silymarin on the adrenal gland in chlorpyrifos intoxicated rats.

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Chlorpyrifos (CPF) is a widely used organophosphorous insecticide that induces toxic effects in man and animal through its inhibitory action on acetyl choline esterase enzyme. The present work aimed to evaluate the toxic effect of chlorpyrifos on the function and structure of the adrenal gland and to investigate the possible protective effect of silymarin antioxidant against such toxicity. The study included twenty four adult male Sprague Dawley rats, which were equally divided into four groups as follows: a control group (n=6) received the oral vehicle only (corn oil), a Silymarin group (n=6) received Silymarin in a dose of 6mg/kg b.w. orally twice weekly for four weeks, the intoxicated group (n=6) received CPF 5mg/kg b.w. orally twice weekly for four weeks and the protection group (n=6) received silymarin orally 6mg/kg b.w. half an hour after chlorpyrifos administration in a dose of 5mg/kg b.w. orally twice weekly for four weeks . By the end of the experiment, estimation of the following biochemical parameters was done: plasma choline esterase enzyme activity level, serum cholesterol, serum cortisol and serum testosterone. The adrenal gland was examined by the light microscope using routine H&E stain and chromaffin stain reaction, as well as by the Transmission Electron Microscope. In the CPF group,ChE, serum cotisol and testosterone levels were significantly decreased compared to the control levels whereas the cholesterol level was significantly higher than the level of control one. Post administration of silymarin after CPF caused improvement of almost all biochemical parameters yet reaching the control levels.

The chlorpyrifos intoxicated group revealed affection of the cells of the adrenal cortex and medulla with variable degrees of degenerative changes. The chlorpyrifos intoxicated group showed that the main histological alteration was the accumulation of large amount of lipid droplets in the cytoplasm of most cells. Ultrastructural examination showed direct degenerative lesion only in few foci within the zona fasciculata. The histological examination of silymarin and CPF group demonstrated a remarkable reduction in the lipid droplets in the cytoplasm compared to CPF intoxicated group. The protection group showed improvement of the levels of the biochemical parameters with partial restoration of the normal histological features of the adrenal structure compared to the CPF intoxicated group. Accordingly, it was proved that silymarin is a reliable antioxidant that could protect against the toxic effect of chlorpyrifos on the adrenal gland.

For Your Notes