Poster Setup and Presentation Times

**Poster Setup**
Sunday, June 25 ........................................... 8:00 AM–12:00 Noon
Your poster must be set up by 12:00 Noon on Sunday, June 25.

**Poster Presentation Times**
*(Please plan to attend your posters during the following times).*
Sunday, June 25 (Welcome Reception) .......... 6:00 PM–6:30 PM
Monday, June 26 ........................................... 9:50 AM–10:20 AM
......................................................... and 3:00 PM–3:30 PM
Tuesday, June 27 ........................................... 9:50 AM–10:20 AM
Wednesday, June 28 ................................... 9:45 AM–10:15 AM

**Poster Teardown**
Wednesday, June 28 ................................... 11:30 AM–1:00 PM
If your poster is not removed before 1:00 PM on Wednesday, June 28, it will be removed and placed near the Registration Desk for pickup.

**Young Investigator Judging Times**
Monday, June 26 ........................................... 7:15 AM–8:00 AM,
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........................................................................ and 3:00 PM–3:30 PM
Tuesday, June 27 ........................................... 9:50 AM–10:20 AM

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**Poster Map (Exhibit Hall)**

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**Exhibitor Area**
*(See page 37 for Exhibitor Map).*

**Entrance**
STP 36th Annual Symposium

Poster Presentation Index

Annual Meeting materials can also be downloaded at www.toxpath.org/am2017/materials.asp or can be viewed via the new mobile app. STP members can access the materials with their normal member login. Nonmember attendees should use login sent via email.

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Subcutaneous Hydrogel Delivery of IL-7 Induces Systemic T Cell Responses and Local Immune Cell Infiltration

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Introduction: Interleukin-7 (IL-7) is critical for T cell survival and is a promising therapy to support T cell responses in cancer therapies and lymphocytopenic conditions. The objective of this study was to evaluate local histopathologic and systemic T cell responses to delivery of IL-7 by a subcutaneous hydrogel.

Experimental Design: C57BL/6NCrl mice were injected once in the flank with hydrogel +/- 200 μg IL-7. The skin/subcutis of the injection site and contralateral side were harvested on days 1, 11, and 30. Axillary/inguinal lymph nodes were collected on these days and with spleens on day 12.

Methods: Skin/subcutis and lymph nodes were evaluated histologically. Cellularity of spleens and lymph nodes was assessed by flow cytometry for CD4+ and CD8+ T cells and lineage CD90.2+ innate lymphoid cells.

Results: Plain gel caused mild, focal, subcutaneous neutrophil infiltration on day 1, followed by infiltrates of predominantly macrophages on days 11 and 30. Gel was nearly cleared by day 30. Large numbers of neutrophils focally infiltrated the subcutis containing IL-7 gel on day 1. On days 11 and 30, necrotic debris, gel, and relatively few neutrophils formed the center of a granuloma rimmed by abundant macrophages and lymphocytes. Flow cytometry demonstrated increased CD4+ and CD8+ T cells and innate lymphoid cells in the spleen and lymph nodes on day 12.

Conclusion: Subcutaneous hydrogel delivery of IL-7 induces systemic T cell responses and localized immune cell infiltration.

Impact Statement: IL-7 hydrogel may be an effective adjunct to T cell therapy.
Carnosine Intervention in the Thy1-aSyn Mouse Model of Parkinson’s Disease

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Parkinson’s disease (PD) is an incurable neurodegenerative disease that affects millions of people worldwide. PD is characterized by motor and non-motor deficits, including gait instability and decreased olfactory function. Molecular hallmarks of PD include protein aggregates and oxidative stress. Our studies evaluated a novel mechanism-based treatment for PD, using the Thy1-aSyn mouse model of PD. Carnosine, an endogenous dipeptide abundant in brain and the olfactory system, declines with age and pathological conditions. In vivo studies indicate that carnosine reduces protein aggregation and protects against oxidative stress, and we confirmed the expression of PEPT2, a carnosine transporter, in olfactory epithelium. Therefore, we hypothesize that intranasal (IN) administration of carnosine will significantly reduce disease progression in the Thy1-aSyn model of PD. Wild-type and Thy1-aSyn mice were treated IN with 2 mg/day carnosine or sterile water for 2 months. Immunohistochemistry, buried food pellet, and the challenging beam traversal (CBT) tests were used to evaluate alpha-synuclein (aSyn) aggregation, and sensorimotor functions. Olfactory function and structure were preserved, and aSyn-positive inclusions were notably lower in the olfactory epithelium of carnosine-treated Thy1-aSyn mice compared to untreated Thy1-aSyn controls. In the CBT test, the number of errors per step was lower in the carnosine treated Thy1-aSyn mice compared to the untreated Thy1-aSyn group (p<0.05). Additionally, the maximal respiratory capacity of mitochondria isolated from the striatum increased in carnosine-treated Thy1-aSyn treated mice compared to controls (p=0.0005). Our findings suggest that carnosine improves mitochondrial function and prevents the progression of motor deficits and aSyn aggregation in the Thy1-aSyn mouse.
Protective Role of Sildenafil against Carbon Tetrachloride-Induced Nephrotoxicity by Augmenting the Availability of Nitric Oxide and Antioxidant Enzymes

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Nephropathy is a leading cause of morbidity and mortality and is characterized by impaired renal function, glomerulosclerosis, and persistent albuminuria, declined glomerular filtration rate (GFR), elevated arterial blood pressure and fluid retention. Carbon tetrachloride (CCl₄) is a colorless, volatile and nonflammable liquid of industrial use and is known to cause hepatotoxicity and nephrotoxicity. The metabolic transformation of CCl₄ results in the generation of free radicals, resulting prominent changes in the morphology of the kidney, including tubulointerstitial fibrosis and vascular congestion. Wistar albino rats of either sex (180-260g), n=6 were employed in present study. Nephrotoxicity was induced by administration of carbon tetrachloride (0.5 ml/kg, s.c.,) for 28 days. Serum creatinine, BUN, urinary microprotein, TBARS, nitrite/nitrate and reduced glutathione estimations were done as hallmarks of renal function. Administration of CCl₄ induced prominent changes in morphology of kidney, including tubulointerstitial fibrosis and vascular congestion, increases in serum creatinine, BUN, urinary microproteins, and renal tissue TBARS levels in comparison to normal control. It also decreased reduced glutathione and tissue nitrite/nitrate levels. Sildenafil treatment (0.4 and 0.8 mg/kg) antagonized the effect of CCl₄ induced renal intoxication dose dependently. L-NAME treatment significantly reversed the effect of Sildenafil treatment. Therefore, it may be concluded from above findings that CCl₄ administration caused marked renal damage. Sildenafil has protective effect in prevention of renal injury by increasing the availability of nitric oxide.
Developmental Toxicity of Trichloroethylene (TCE) in Zebrafish (Danio rerio)

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Introduction: Trichloroethylene (TCE), an industrial degreaser and solvent, is an environmental toxicant that contaminates over half of Superfund sites. TCE, a known carcinogen, is also linked to congenital defects and neurodegenerative disease. The US EPA maximum contaminant level of TCE in drinking water is 5 ppb (parts per billion); however, ground water levels can be >10,000 ppb at Superfund sites. 

Objective: The developmental toxicity of TCE near regulatory levels needs further characterization in order to better assess risk. 

Experimental Design: In this study, the zebrafish model was used to evaluate the acute developmental toxicity of near regulatory concentrations of TCE by monitoring survivability, percent hatching, morphological measurements, and neurobehavior. 

Materials and Methods: Zebrafish embryos were dosed immediately after fertilization with 0, 5, 10, 50, or 500 ppb TCE, or 0.5 ppb 1-trichloromethyl-1,2,3,4-tetrahydro-β-carboline (TaClo), a TCE metabolite. Embryos were exposed through 72 hours post fertilization (hpf) rinsed, and kept in control water until 120 hpf. 

Results: The percent survival and hatching were not significantly different between treatment groups (p > 0.05). Morphological measurements indicated that the 500 ppb TCE, 10 ppb TCE, and 0.5 ppb TaClo groups had significantly shorter body lengths compared to the controls (p < 0.05). The 500 ppb TCE group had significantly shorter brains (p < 0.05). No significant differences were observed during the evaluation of neurobehavior (p > 0.05). 

Conclusion and Impact: The morphologic alterations suggest that developmental TCE toxicity is still a concern near regulatory concentrations and that TCE should remain a priority environmental toxicant.
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Damage-Associated Molecular Patterns (DAMPs) and MHC Class II-Expressing Cells in Thioacetamide (TAA)-Induced Rat Liver Injury

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Introduction: DAMPs, which are released from injured/necrotic cells, and their ligand-receptor, MHC class II and Toll-like receptors (TLRs), play important roles in the pathogenesis of chemical-induced liver injury. To elucidate their functional roles, we analyzed the kinetics of these factors and related cells in TAA-induced rat liver injury. Experimental Design: 1) Acute liver injury: F344 rats (male, 7 weeks) were injected with TAA (300 mg/kg BW, i.p.) and liver samples were collected from 10 hours to day 7 post injection. 2) Repeated-injection liver injury: F344 rats (male, 7 weeks) were injected with TAA (100 mg/kg BW, i.p.) one to three times with three-day interval and samples were collected on 2 days post each injection. Results: 1) Coagulation necrosis developed in perivenular areas on day 1, followed by inflammatory cell reaction on days 2 and 3. MHC class II+ cells and DAMPs expressions peaked on day 2, and TLR4 expression peaked on day 3. 2) After the first injection, hepatocyte injury was seen, accompanied by inflammatory cell reaction; however, after the twice and thrice injections, the injury level was reduced. AST and ALT values were decreased in repeated injections. Expression of FMO3, a TAA metabolizing enzyme, was decreased in repeated injections. Impact Statement: 1) DAMPs-TLR4 axis may be the key pathway in hepatotoxicity. 2) Hepatocytes may come to avoid TAA injury via cytoprotective functions.
A New “Old” Approach to Analyse the Rat Fetus and Placenta at 19.5 Days of Gestation

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Introduction: Histopathological examination of the placenta and fetus is rarely done in rats. Toxicological studies generally rely on gross examination of the fetus and placental weight. The small size of the fetus, however, makes its dissection labor-intensive. Furthermore, placental weight is insufficient to assess the toxicity of xenobiotics since the placenta is a frequent target. Thus, our objective was to develop a simple and accurate technique for evaluating the rat fetus and placenta.

Methods and experimental design: Sprague-Dawley rat fetuses at GD19.5 and their placentas were collected, weighed (n=98) and fixed whole (n=32 and n=18, respectively) in formalin. The placentas were cut transversally in the center. Fetuses were first decalcified and then cut following a free-hand whole-body serial sectioning scheme adapted from Wilson's method (1965). Sections were embedded and stained with HEPS. Histomorphometry was used to measure the area of each fetal placental region.

Results: The average placental and fetal weights were respectively 0.50±0.05g and 3.0±0.3g, with a placental:fetal ratio of 0.15±0.02. Fetal placental region area was 27.2±1.7mm\textsuperscript{2}, including the labyrinth (22.2±1.0mm\textsuperscript{2}) and the basal zone (4.8±0.8mm\textsuperscript{2}). Our whole-fetus serial sectioning scheme resulted in 12 precise histological slides enabling the examination of most organs without labor-intensive dissection.

Conclusion: Performing histopathological examination of fetuses using this whole-body serial sectioning method is more efficient than individual organ dissection. Quantitative analysis of the placental areas improves understanding of the pathogenesis of treatment-related changes. Impact statement: This technique provides a standardised method for future research in pertinent fields such as development and toxicology.
Folic Acid As an Answer to the Impact of Arctic Contaminants on Inuit: What Is the Effect on the Father and the Health of His Offspring?

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Introduction: Because of their traditional diet, Inuit people are 40X more contaminated in Persistent Organic Pollutants (POPs) than non-aboriginal Canadians. Moreover, Inuit diets are deficient in folic acid (FA). Those factors could partly explain why Inuit are subject to more perinatal defects and chronic circulatory diseases and have a life expectancy nearly 15 years shorter. Since it has been recently shown that the father's lifestyle also influences the health of his offsprings, our hypothesis is that FA supplementation could attenuate the phenotypic effects observed following paternal prenatal exposure to POPs. Methods and experimental design: Sprague-Dawley rat females (F0) were gavaged for 5 weeks before and throughout gestation with corn oil (control) or an environmentally-relevant mixture of Arctic POPs. These females (n=6) received either a basal dose of FA (1X) or a FA supplementation (3X). Their F1 male pups (n=12) underwent necropsy and blood sampling at PND150. Results: Kidney (p=0.01) and brain (p=0.09) weights were increased with POPs. Blood platelet count and hematocrit were decreased by POPs, a change which was corrected by FA supplementation (p=0.01). Erythrocyte counts were higher with FA supplementation (p=0.05). Conclusion: Those results suggest that prenatal POPs and FA exposure alter the development and function of certain organs. Also, the altered blood parameters could be representative of the high rates of circulatory diseases observed in Arctic Inuit populations. Impact statement: Achieving our objectives will broaden current understanding of the toxicological impacts of the environment on human health and the developmental origins of disease.
Evaluation of Microcalcification in Cardiovascular Medical Devices

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The current FDA draft guidance “General Considerations for Animal Studies for Medical Devices” encourages the use ex vivo imaging modalities such as radiography, micro-computed tomography (microCT), and histomorphometry to identify adverse calcification in medical devices. Here, we describe the use of these modalities to characterize the presence or absence of device microcalcification in two chronic ovine (Ovis aries) surgical models. Sheep were implanted with either a tissue engineered vascular graft (TEVG, an interposition graft in the thoracic caudal vena cava) or a novel transcatheter pulmonary valve device (MZ-TPV, for the treatment of pulmonary regurgitation). In vivo, 2D and 3D rotational angiography were utilized while ex vivo methodology included microCT, radiography, and histology. Early device calcification was identified using MicroCT (TEVG) and radiography followed by histomorphometry (MZ-TPV, including the ability to localize microcalcification to one valve leaflet). Each of the modalities used had unique strengths (better 3D special resolution for microCT; lower cost of use for radiography/histomorphometry) and could be used alone or in combination (e.g. identifying individual micocalcification foci with imaging helps optimize tissue-sampling for histomorphometry). In conclusion, the utilization of ex vivo imaging modalities can serve to further inform interpretation and assessment of cardiovascular device studies.
Insights into the Effect of Subtle Chemical Modification of Aryl Chroloethylurea (CEU) As Small Molecules Targeting Either β-Tubulin or Prohibitin and Thioredoxin-1

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Introduction: Chloroethylureas (CEU) are protein alkylating agents displaying potent antineoplastic properties that covalently bind to β-tubulin and affect microtubule polymerization dynamics. A different CEU subset has been shown to induce cell growth inhibition without alkylating β-tubulin. Our research focus is to understand the mechanisms underlying the antiproliferative activity of that new class of compounds. Methods: Proteins from B16 and MDA-MB-231 cells incubated with [(14)C-urea]-CEU-25 and [(125)I]-CEU-98 were separated using 2D-electrophoresis followed by MALDI-TOF identification of modified proteins. Protein expression and distribution were investigated by Western blot analyses and immunocytochemistry. Cell cycle analyses were obtained by flow cytometry. Results: CEU-22 and its bioisosteric derivative CEU-98 are original CEU prototypes that covalently bind to β-tubulin via an ester linkage on Glu198. The alkylation leads to microtubule depolymerization phenotype, cell cycle arrest in G2/M and inhibition of cell proliferation in vitro. A newly isolated subset of CEUs, exemplified by the prototypical CEU-25, alkylates prohibitin (PHB) on Asp40 and thioredoxin isoform-1 (TRX1). CEU-25 arrests cells predominantly in G1 phase and inhibits Trx-1 and PHB nuclear translocation. Conclusion: The intracellular proteins alkylated by the new CEU subset were identified as the PHB and TRX1. Different protein target profiles explain the G1 cell cycle arrest. Our research emphasizes that a subtle chemical modification might lead to drastic change of protein target. Impact statement: Our finding might help to design new potent anticancer drugs that will target specific and lethal biological pathway essential to tumor growth.
Diminished microRNA-29b Results in Overexpression of BRD4 and BRD4-Related Oncogenes in Cutaneous T-Cell Lymphoma

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1Ohio State University, Columbus, OH, United States, 2Harvard Medical School, Boston, MA, United States, 3Thomas Jefferson University, Philadelphia, PA, United States

Introduction: Cutaneous T-cell lymphoma (CTCL) is a malignancy of mature CD4+ T-cells that initially presents in the skin but may progress and spread systemically. Molecular hallmarks of disease progression include altered expression of microRNAs (miRs) and epigenetic dysregulation. Here, we characterize aberrant epigenetic modifications in malignant T-cells that contribute to CTCL development and progression. Experimental Design: Initial mechanistic work was performed in CTCL patient-derived cell lines. BRD4 binding was confirmed in purified CD4+ T-cells from CTCL patients via chromain immunoprecipitation-sequencing. The proposed pathway was validated using interleukin-15 (IL-15) transgenic mice that develop CTCL by 8 weeks of age, which were treated with bromodomain and extra-terminal motif (BET) inhibitor JQ1, or proteasome inhibitor bortezomib. Methods: RT-PCR was performed for miR-29b and target genes. Immunoblotting was performed utilizing actin as an internal control. Mouse tissues were assessed histologically and immunohistochemically. Results: miR-29b levels are significantly decreased in CTCL patients compared to healthy donors. BET protein BRD4, which is regulated by miR-29b, is bound extensively at promoter sites in patients but not in healthy donors. Disruption of BRD4 binding by BET inhibitor JQ1, or rescue of miR-29b level by bortezomib, halts progression of CTCL in IL-15 transgenic mice. Conclusion: BRD4 binding results in increased expression of lymphoma-associated proteins NOTCH1 and RBPJ, as well as increased expression of all three components of the IL-15 receptor complex; the latter enhancing the IL-15 autocrine signaling loop that augments CTCL progression. Impact statement: We describe a novel targetable oncogenic pathway featuring IL-15, miR-29b, and BRD4 in CTCL.
Incidence and Age Association of Increased Stromal Collagen in Testes of Cynomolgus Monkeys

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Introduction: Cynomolgus macaques (Macaca fascicularis) are commonly used in toxicity studies. Consistent and accurate identification of background findings in toxicity studies is important for evaluation of potential toxicities. The aim of this study is to characterize the epidemiological incidence of a common background finding, increased stromal collagen, or fibrous hypoplasia, of the testes in male cynomolgus monkeys. The etiology of this finding is currently unclear and may be due to sectioning variability, increased collagen, or replacement of seminiferous tubules with fibrous tissue. Experimental Design and Methods: We performed retrospective histological examination of testes from all animals in 43 recent studies. Testes of 660 (157 control and 503 dosed) animals were graded on maturity and degree of increased collagen. Body weights, reproductive organ weights, and age from control animals were evaluated for statistical correlations. Results: Incidence rates were similar between control and dosed animals. Immature animals had the highest incidence (51%) of increased stromal collagen of the testes. There was a statistical correlation with age, with immature animals affected significantly more often than mature animals (p<0.05). Conclusion: Increased stromal collagen is a common spontaneous finding in the testes of cynomolgus monkeys, occurring primarily in immature animals, with incidence decreasing in mature animals, although sample size, trimming patterns, and relative ease of detection in immature animals may impact the apparent incidence. Impact statement: This finding occurs commonly with a similar incidence in control and dosed animals and is most commonly detected in immature macaques.
Vaccination of Mice with VC2, a Novel Mutant Strain of Herpes Simplex Virus 1, Protects Against Ocular Herpesvirus Infection

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Introduction: Recurrent activation of human simplex virus-1 (HSV-1) results in cold sores, periodic viral shedding in tears and herpetic stromal keratitis in humans. We evaluated the efficacy of a novel mutant herpes virus VC2 as vaccine against mouse ocular herpes models, and characterized the immunological correlates of protection. Experimental Design: Mice were vaccinated twice at 21-day intervals by intramuscular injection, prior to ocular HSV-1 challenge. Cornea, lymph nodes, spleen and serum were analyzed on 5, 10 and 15 days post-infection. VC2 vaccine group was compared with live-attenuated HSV-1 (F) viral vaccine, and naïve group. Methods: Immunohistochemistry and confocal microscopy were used to assess the features of keratitis. Serum antibody levels were measured against HSV-1 by ELISA and serum neutralization assay. Flow cytometry of lymph nodes and spleen, and cytokines levels in cornea and serum were assessed. Results: VC2 vaccine reduced the lethality rate and protected mice from developing clinical keratitis upon ocular HSV-1 challenge. VC2 produced a prominent IgG2a type response while mice vaccinated with HSV-1(F) produced a significant IgG2b type response. Mice vaccinated with live attenuated HSV-1(F), unlike VC2, showed elevated levels of total systemic CD4+ T cells and persistence of ocular disease. Conclusion: Immunization with our novel mutant strain VC2 provided complete protection against ocular herpes infection in mice, and elicits altered humoral and cell immune response compared to the live-attenuated wild type HSV-1 strain. Impact statement: Based upon our evaluation in mouse models, VC2 vaccine has a strong potential to protect against ocular herpes infection in humans.
An Immunohistochemical Investigation of Renal Phospholipidosis and Toxicity in Rats

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Objectives: The objective of this study was to use immunohistochemistry to determine the relationship between colocalization of renal tubular phospholipidosis and proximal tubular expression of KIM-1 as an in situ biomarker of renal tubular injury. Experimental Design: SD rats (20/sex/group) were administered vehicle or a novel drug candidate known to induce phospholipidosis in rats. Following a dosing period of 6-months, kidney tissues were harvested at necropsy, sectioned and stained with H&E. Immunohistochemistry for KIM-1 (renal tubular injury marker), LAMP-2 (marker of phospholipidosis) and adipophilin (fat) was performed. Results: H&E staining revealed renal tubular cell vacuolation, hypertrophy, degeneration and dilation of renal tubules in rats administered the test article. Renal tubular injury was confirmed using KIM-1 IHC. The presence of phospholipidosis was confirmed by positive staining for LAMP-2. The increased LAMP-2 staining was most prominent in the outer stripe of the outer medulla (OSOM) co-localizing with site specific expression KIM-1. By contrast, adipophilin staining was not increased. Phospholipidosis also was confirmed by electron microscopy. Conclusion: Statistically significant increased LAMP-2 and KIM-1 labeling was observed in the OSOM tubular epithelial cells in the test article group rats compared to the control rats (p<0.001). Although co-localized KIM-1 and LAMP-2 immunostaining indicated an association between phospholipidosis and renal toxicity, a causal relationship could not be established. Impact statement: These data support the use of LAMP-2 IHC as a diagnostic tool, and suggest an association between phospholipidosis and renal tubular injury.
Detection and Correlation of Kidney Safety Biomarker (Kim-1 and Clusterin) in Urine and In Situ Expression of Kim-1 and Clusterin in Kidneys from Renal Toxicity Model Using Gentamicin or Cyclosporine in Rats

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Introduction: Understanding how kidney safety biomarkers correlate with drug-induced renal histomorphologic changes and expression of these markers in situ are important for their preclinical or clinical application. This study was conducted to investigate the correlation between urine Kim-1 and clusterin levels and in situ expression of these biomarkers at both RNA and protein levels in rat kidneys with drug-induced injury. Experimental Design: Male rats [Crl:CD (SD) 7-11 weeks old] were given a daily oral dose of vehicle, gentamicin (80 mg/kg) or cyclosporine (30 or 60 mg/kg), and were necropsied on Days 3, 9 or 15 (n=5/dose/necropsy). Methods: Kidneys were fixed, stained with H&E and examined histomorphologically. Urine Kim-1 and clusterin were detected with ELISA. In situ expression of Kim-1 and clusterin was detected using immunohistochemistry (IHC) or ViewRNA methods (in situ hybridization [ISH]). Results: Treatment-related tubular changes were observed in the gentamicin-treated rats beginning Day 3, and at 30 and 60 mg/kg in cyclosporine-treated rats beginning Day 9. In IHC and ISH, protein and mRNA of Kim-1 or clusterin often colocalized in affected tubules. Increases in mRNA and protein of Kim-1 or clusterin in focal tubular epithelial cells were observed in several rats where drug-related changes were not evident in H&E sections. Conclusion: The elevation of urine Kim-1 and clusterin levels correlated with the presence of drug-related renal histomorphologic lesions and with in situ expression of Kim-1 and/or clusterin. Impact statement: This work supports the use of urine biomarkers (Kim-1 and clusterin) for a sensitive detection of drug-induced renal injury.
Gastric Cryptosporidiosis in Immunocompetent Cynomolgus Macaques (*Macaca fascicularis*)

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We report two cases of Cryptosporidium species identified in the stomachs of immunocompetent, asymptomatic cynomolgus macaques. Cryptosporidium muris-like organisms have been reported in stomachs of immunocompromised cynomolgus macaques; however, this is the first report of infection as a background finding in monkeys without evidence of immune suppression. A complete necropsy was performed on two cynomolgus macaques (*Macaca fascicularis*) from control groups in their respective studies. The cardia, body, and pyloric sections of the stomach were fixed in 10% neutral buffered formalin and stained with hematoxylin and eosin (H&E) for routine light microscopy. Toluidine blue, acid fast, Periodic Acid Schiff (PAS), Giemsa and nested PCR were used for further analysis. Intracellular, extracytoplasmic organisms of varying life stages consistent with Cryptosporidium sp. were present along the luminal surface of the upper third of the gastric glands in the body of the stomach with enlargement of mucous epithelial cells. These organisms were identified based on the H&E and special histochemical staining characteristics. The PCR did not detect cryptosporidia, which was considered most likely due to degradation of nucleic acids due to the fixation process. In conclusion, Cryptosporidium sp. can infect cynomolgus macaques regardless of immune status and without eliciting clinical signs. This organism can also infect various other laboratory species that are used in nonclinical research, a review of which will be provided. It is important that toxicologic pathologists are familiar with cryptosporidiosis in cynomolgus macaques as it can cause morphologic changes in tissues which could be mistakenly attributed to test article administration.
Analyst and Smear Variability in Cytological Bone Marrow Cell Differential Counts and Assessment of Full Differentials and Proliferating and Maturing Cell Stage Differentials

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Introduction: Results from cytological bone marrow cell differential counts in nonclinical toxicology studies may be affected by biological, procedural, or analyst-related variability. Experimental Design: Cytological bone marrow cell differential counts were performed on smears from cynomolgus monkeys to evaluate inter-analyst and intra-smear variability. Methods: Differentials were performed on the same five fields by three clinical pathologists (CPs); one CP evaluated one animal five times across three smears; and three CPs independently evaluated 24 animals to assess inter-analyst (across fields), intra-smear, and inter-analyst (across animals) variability, respectively. Intra and interclass correlation coefficients were used to determine agreement for each cell type, myeloid to erythroid (M:E) ratios, and calculated proliferating and maturing myeloid and erythroid stages. Results: Most cell types had moderate or excellent agreement for intra-smear and inter-analyst (across animal) evaluations. Inter-analyst (across fields) agreement was typically moderate or excellent for cell types with higher percentages, while cell types with low percentages in the marrow had poor or no agreement. Lymphocytes had poor agreement across all analyses. Proliferating and maturing cell stages had moderate to excellent agreement for inter-analyst (across animals) variability. Conclusion: Agreement was moderate or excellent for most differentiated cell types across analyses. Both inter-analyst and intra-smear variability contributed to poor lymphocyte agreement. Using proliferating and maturing pool differentials was supported by moderate to excellent agreement. Impact statement: Full differential and proliferating and maturing stage differential endpoints typically had moderate to excellent agreement, and either scheme may be appropriate for cytological bone marrow evaluation in nonclinical toxicity studies.
Preclinical *In Vitro* and *In Vivo* Safety Evaluation of Cinnamon Extract

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Cinnamon bark is commonly used for herbal therapy and food ingredients in Korea. Most Cinnamon bark is imported from Vietnam. However, repeated toxicity and genotoxicity study for Cinnamon extract was not carried out yet. Therefore, this study was performed to evaluate 13-week repeated dose toxicity (13-RDT) and genotoxicity study according to the KNTP protocol and GLP guideline.

In preliminary studies, Cinnamon extract was not toxic response up to 2000mg/kg B.W. in 14-day repeated dose toxicity study. Also, Genotoxic potency was not observed at any doses in bacterial reverse mutation assay, *in vitro* chromosome aberration assay and *in vivo* micronucleus test. Based on preliminary results, Cinnamon extract was orally administered five times per week for 13 weeks in F344 rats at dosage levels of 0, 25, 75, 225, 665, and 2000 mg/kg/B.W. Vehicle control group were received D.D.W.

No test substance-related effect were observed in all criteria included mortality; clinical signs; body weight changes; food and water consumption; hematology and serum biochemistry parameters; male and female reproductive tissue evaluations; gross findings and histopathological findings. There were not found any target organ in histopathological findings.

Therefore, NOAEL for Cinnamon extract might be thought 2000 mg/kg in males and females.
**Short-Term Toxicity Evaluation of Aloe Vera Whole-Leaf Freeze Dried Powder in SD Rats**

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Introduction: Being a widely used natural herb, Aloe vera has derived many sorts of commercial products with different preparation technologies, and aloe whole-leaf powder is one of the most popular subtypes. So far, the long-term impact of aloe products has already been reported, however, there were little studies concerning about the short-term exposure, especially with Aloe Vera Whole-leaf Freeze-dried Powder (AWFP). Experimental Design: 120 SD rats were divided into four groups (Control and 400/1200/2000 mg/kg treatment groups), and were administered with AWFP once daily by oral gavage for consecutive 28 days with a 2-week recovery phase. Methods: Measurements such as in-life observations, body/organ weight as well as hematology/biochemistry detection, histopathology with semi-quantitative evaluation were employed to constitute the whole evaluation panel. Results: AWFP induces soft/loose changes of feces; meanwhile, significant decreased WBC associated with reduced lymphocytes could be noted. The relative organ weight including organ to body weight ratio and organ to brain weight ratio of kidneys were significantly increased in 2000 mg/kg compared with controls. Histopathologically, pigmentation in kidneys and increased mucosal thickness in colon were also noted in dose groups; 400 mg/kg was considered as the NOAEL level. Conclusion: The study provided clear evidence of treatment-related changes with a short-term exposure of AWFP. Impact statement: This is the first time reporting the early colon morphologic changes associated with stool changes noted in the in-life phase, which provided more toxicity data to the short-term usage of AWFP as a remedy.
Rodent Hepatocellular Tumor Responses in the National Toxicology Program (NTP) Bioassays

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Introduction: Hepatocellular tumors are the most common treatment-related tumors in both rats and mice in the National Toxicology Program (NTP) bioassays. It is assumed that the high background rate of liver tumors in the B6C3F1/N mouse (72% male and 35% female) is responsible for even higher liver tumor incidences after chemical exposures and that the mouse model may over predict carcinogenicity. Materials/Methods: Hepatocellular tumor responses were analyzed from 490 NTP bioassays where the same chemical was tested in both rats and mice from 13-week/subchronic and 2-year/chronic studies. Results: 146 bioassays had a treatment-related hepatocellular tumor response in rat only (10%), mouse only (65%), or both species (25%). Hepatocellular tumors with a clear or some evidence of carcinogenicity were observed at 29% in both sexes of F344/N rats and 62% and 80% in male and female B6C3F1/N mice, respectively. Liver tumor only response in mice with no tumors in other sites or in rats accounted for less than 15% of the tested carcinogens while over 85% were multi-site and/or dual species carcinogens. Only 25% of the liver carcinogens had a positive response in Ames/Micronucleus assay. Conclusion: Male mice have lower treatment-related liver tumor response after chemical exposures than female mice despite high background incidence. Majority of the liver carcinogens may have a non-genotoxic mode of action. Impact Statement: Mouse liver tumor responses do not seem to over predict carcinogenicity since most of the mouse liver carcinogens also resulted in tumors in other organs and/or in rats.
Uterine Subserosal Cysts in Harlan Sprague-Dawley Rats from National Toxicology Program Studies

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Introduction: Cystic endometrial hyperplasia and endometrial cysts are two of the most common lesions of the aging rat uterus. However, cysts involving the subserosal surface of the rat uterus are uncommon. Experimental Design: Spontaneous subserosal uterine cysts were observed in 20 out of 2,400 female Harlan Sprague Dawley rats evaluated in five multi-generation studies conducted by the National Toxicology Program (NTP). Results: Subserosal cysts were recognized grossly as unilateral, small fluid-filled, clear cysts or nodules, which occurred along the length of the uterine horn. Microscopically, cysts were associated with a well-developed smooth muscle wall, lined by flattened to cuboidal epithelium that stained intensely positive for cytokeratin-18 and weakly positive for estrogen receptor alpha and progesterone receptor. Cyst lumens contained lightly basophilic staining flocculent material. Conclusion: The origin of the cysts appeared to be developmental anomalies postulated arising from Wolffian or Müllerian duct remnants. Impact statement: Based on our findings, we have identified developmental subserosal uterine cysts in rats that have not been extensively characterized in the rodent pathology literature.
Heterotopic Ossification in the Achilles Tendon (Calcanean Tendon) of the NOD/scid/Il2rY-/- Mouse

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Introduction: A colony of humanized immunodeficient mice (NOD/scid/Il2rY-/- mice) were established for upcoming immunological studies. Approximately 22 out of 56 mice were clinically noted to have unilateral or bilateral soft swelling of the tibio-tarsal joint (hocks). A total of 24 out of 112 legs were affected with this condition, which had not been previously observed in conventional mice in our colony. Our objective was to investigate the cause of the swelling. Methods and Materials: Necropsies were performed on 5 animals, and samples were submitted for microbiological culture and PCR. Affected and unaffected hocks from 5 animals were fixed in 10% neutral buffered formalin, decalcified, sectioned and stained with hematoxylin eosin-phloxine and Twort’s Gram stain. Results: In affected hocks, the Achilles tendon was thickened up to approximately 4X the unaffected tendon, by the presence of woven bone and fibrous stroma affecting the distal 2 mm of the tendon extending to the periosteum of the calcaneous, compatible with heterotopic ossification. Microbiological culture of affected lesions yielded no growth, was negative on PCR and no microorganisms were visible microscopically. Conclusions: Heterotopic ossification in NOD/scid/Il2rY-/- mice has not been described in the literature. Our findings suggest that this strain of mice may be susceptible to tendon ossification. Heterotopic ossification may develop secondary to traumatic injury, musculoskeletal diseases, pharmacologic agents or genetic disorders. Impact statement: Genetically modified mice may be susceptible to unexpected changes that have no obvious relationship to the genetic background, some of which may be confounding variables in testing and research.
Ninety-Day Toxicity and Single-Dose Toxicokinetics Study of Alpha-Glycosyl Isoquercitrin in Sprague-Dawley Rats

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Introduction: alpha-Glycosyl isoquercitrin (AGIQ) is a highly absorbable mixture of isoquercitrins with antioxidative properties. It has been confirmed as a generally recognized as safe (GRAS) compound by the FDA, and approved by the Japanese Ministry of Health and Welfare for use as a food additive. Nevertheless, safety and toxicity information for AGIQ is still sparse and outdated. Objectives: To comprehensively evaluate the toxicity of AGIQ in Sprague-Dawley rats exposed orally for 90 days and to explore the toxicokinetics (TK) of AGIQ. Experimental Design: 60 male and 60 female Sprague-Dawley rats were administered AGIQ daily at dietary doses up to 5%, and followed for 90 days. TK of AGIQ was tested in twenty male Sprague Dawley rats up to 24 hours following a single gavage dose. Results: All animals survived with no signs of morbidity, and no evidence of systemic toxicity. AGIQ was rapidly absorbed with metabolism to quercetin and quercetin glucuronide at all dose levels. Dose dependent yellow discoloration of bones was observed, but no changes were found microscopically, and this observation was concluded as toxicologically insignificant. Conclusion: The overall lack of adverse clinical signs, changes in body weight, feed consumption, clinical pathology parameters, and histopathological endpoints in animals administered AGIQ supports no observable adverse effect levels (NOAEL) of 5.0% in diet for both male and female rats. Impact statement: Based on this study, AGIQ can continue to be safely used as a food additive.
INHAND: International Harmonization of Nomenclature and Diagnostic Criteria for Lesions—An Update—2017

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The INHAND Proposal (International Harmonization of Nomenclature and Diagnostic Criteria for Lesions in Rats and Mice) has been operational since 2005. A Global Editorial Steering Committee (GESC) helps coordinate overall objectives of the project. Development of harmonized terminology for each rodent organ system or non-rodent species is the responsibility of the Organ Working Groups (OWG) or Non-rodent Working Groups (NRWG) respectively, drawing upon experts from North America, Europe and Japan.

Great progress has been made with 10 rodent organ systems published to date – Respiratory, Hepatobiliary, Urinary, Central/Peripheral Nervous Systems, Male Reproductive and Mammary, Zymbals, Clitoral and Preputial Glands in Toxicologic Pathology and the Integument and Soft Tissue, Female Reproductive System, Digestive System, Cardiovascular System and Skeletal System in the Journal of Toxicologic Pathology as supplements and on a web site – www.goReni.org. Recommendations of the Apoptosis/Necrosis Working Group have been published. INHAND guides offer terminology, diagnostic criteria, differential diagnoses and guidelines for recording lesions in toxicity and carcinogenicity studies. The guides provide representative photo-micrographs of morphologic changes, information regarding pathogenesis, and key references.

INHAND GESC representatives attend meetings with representatives of FDA Center for Drug Evaluation and Research (CDER), Clinical Data Interchange Standards Consortium (CDISC), and National Cancer Institute (NCI) Enterprise Vocabulary Services (EVS) to assist with incorporating INHAND terminology as preferred terminology for SEND (Standard for Exchange of Nonclinical Data) submissions to the FDA. Interest in INHAND nomenclature, based on input from industry, government toxicologists and information technology specialists, is encouraging wide acceptance of this nomenclature.
Common Spontaneous Histopathologic Findings in Juvenile Domestic Pigs Used in Nonclinical Research Studies

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Introduction: Juvenile pigs often serve as the most suitable non-primate animal model for human pediatric nonclinical research (e.g. nutritional studies) based on their similar physiologic and anatomic characteristics. Knowledge of the types of spontaneous (background) microscopic findings in young pigs is important to distinguish incidental from test article-related effects; however, currently there is a paucity of publications on this topic. This poster presents microscopic historical control data for 108 juvenile (23-24 days old) domestic Yorkshire crossbred (farm) piglets over a 5 year period at a nonclinical contract research organization. Methods: Microscopic and organ weight historical control data from a total of 108 control Domestic Yorkshire Crossbred (farm) piglets of 23-24 days of age (54 males and females) was compiled. Results: Microscopic findings of highest incidence rates included: mononuclear cell infiltrates in various tissues (liver, brain, heart, and kidneys); stomach hyperkeratosis, bacteria, inflammation, and erosions; intestinal inflammation, glandular dilation, and degeneration; extramedullary hematopoiesis in the liver and spleen; pleural adhesions/inflammation/fibrosis; and renal fibrosis, tubular basophilia/regeneration, developmental anomalies, cysts, transitional cell hyperplasia, tubular dilation, and pyelonephritis. Polyarteritis was also infrequently noted in various tissues. Conclusion: Spontaneous (background) microscopic findings in juvenile pigs occur and may confound interpretation of test article-related findings. Impact statement: Presented information provides a reference for the types of background lesions that occur in juvenile pigs and may be beneficial to pathologists to discern spontaneous versus test article-related effects.
Toxicity and Pharmacodynamics of Two CLL-1 T Cell Bispecific Antibodies with Varying CD3 Affinity

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Introduction: Acute Myeloid Leukemia (AML) is an unmet medical need for which a T-cell dependent bispecific antibody (TDB) is a potential therapeutic. C-type Lectin-like molecule 1 (CLL-1) is a promising target for such a TDB as it is expressed in AML, but not on normal hematopoietic stem cells. We compared the toxicity and pharmacodynamics of two TDBs with the same anti-CLL-1 arm and either a low affinity anti-CD3 arm (aCLL1-L) or a high affinity anti-CD3 arm (aCLL1-H). Experimental design: Male cynomolgus monkeys received vehicle (n=3), 0.5 mg/kg aCLL1-H (n=3), 0.5 mg/kg aCLL1-L (n=3), or 0.2 mg/kg aCLL1-L (n=6) by IV infusion and were observed for 8-28 days. Methods: Hematology, T-cell activation status, serum cytokines, clinical chemistry, coagulation, and toxicokinetics were assessed. Bone marrow CLL-1+ cells (flow cytometry) and histopathology were conducted at necropsy on D8, D21 or D28. Results: 0.5 mg/kg aCLL1-H resulted in severe toxicity and morbidity within 8-36 hours associated with massive cytokine release, T-cell activation, and microscopic evidence of vascular collapse. Administration of 0.5 mg/kg aCLL1-L resulted in febrile neutropenia in one animal requiring euthanasia on D6. Administration of 0.2 mg/kg aCLL1-L was well tolerated, inducing modest elevations of serum cytokines, marked depletion of target cells by D4-8 that recovered by D12-15, and reversible hypocellularity in the bone marrow and lymphoid tissues. Conclusion: Altering the affinity of the anti-CD3 arm of TDBs can dramatically alter their toxicity. Impact statement: aCLL1-L resulted in optimal pharmacodynamics with minimal toxicity in monkeys and could be effective in AML.
Variance in Regional Skin Histoanatomy in Göttingen Minipigs

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Introduction: Miniature swine, including Göttingen minipigs, are increasingly used in biomedical research and are the preferred non-rodent species in preclinical dermal safety studies. Minipig integument has been reported to have morphologic features and percutaneous absorption similar to human skin. Furthermore, like human skin, the histomorphology of porcine skin varies based on anatomic location. Careful consideration of regional anatomic differences in cutaneous histomorphology must be accounted for in the discernment of compound-related effects, however there is a paucity of data regarding characterization of skin by body region in Göttingen minipigs. In this study, we characterized regional histomorphologic variation utilizing quantitative and semi-quantitative assessment of epidermal, dermal, and subcutis thickness; stratum corneum thickness; and density of hair follicles, sebaceous glands, and apocrine glands.

Methods and Materials: Six Göttingen minipigs were euthanized and skin was systematically collected from multiple sites. Experimental Design: We mapped multiple skin sites, including common dermal application sites, and systematically sampled 3 cm² sections of each site, characterizing differences in histoanatomy. Results: Significant variation in the thickness of the dermis and subcutis and density of sebaceous and apocrine glands were identified in the dorsum, ventrum, inguinal region, and limbs. Slight regional variations in stratum corneum thickness were also observed, whereas overall epidermal thickness generally did not vary between sites. Conclusion: There is significant regional variability in the microscopic anatomy of the skin in Göttingen minipigs. Impact Statement: Regional histomorphologic differences must be accounted for to avoid misinterpretation of test article-related effects in dermal safety studies in Göttingen minipigs.
Case Report: Extramedullary Hematopoiesis in Canine Pituitary Glands

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Background: Extramedullary hematopoiesis (EMH) is the formation and development of blood cells outside the medullary spaces of the bone marrow. EMH in spleen, lymph nodes, liver, adrenal glands, mammary gland and choroid plexus have been reported in beagle dogs, however, we found no reports of EMH in the pituitary gland. In this case report, we document EMH in pituitary glands of beagle dogs on safety assessment studies; all dogs were 7-10 months old. Case presentation: 6 dogs (3 males and 3 females from control groups) had extramedullary hematopoiesis in the pituitary gland without associated clinical pathology or macroscopic abnormalities. In each case, microscopic examination showed multiple foci of hematopoietic cells consisting of predominately erythroid precursors (metarubricytes, prorubricytes, rubricytes, rubriblasts), often with modest numbers of promyelocytes; all foci were in the pars distalis.

Discussion: This report of EMH in the pituitary gland of beagle dogs from control groups, provides literature documentation that can help substantiate the presence of EMH in treated groups as possibly incidental background findings with no test article association.
Proliferative Cartilaginous Lesions in the Tibiotarsal Joints of HuNOG Mice

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Introduction: HuNOG mice (NOD/Shi-scid/IL-2Rγnull, or NOG, mice humanized with CD34+ hematopoietic cells) are used in engraftment studies due to their ability to accept heterologous cells. The purpose of this study was to report unusual lesions affecting the tibiotarsal joints of huNOG mice that were used in a study to evaluate proprietary immune-modulating drugs. Experimental Design: Two of nine 26-week old huNOG mice developed unilateral swelling of one tibiotarsal joint, associated with paresis, during a three-week study. Full necropsies, including examination of affected tibiotarsal joints, were performed after euthanasia at the experimental endpoint, which was one week after the onset of clinical signs. Methods: Routine tissues and affected tibiotarsal joints were processed for histopathologic analysis. Results: Both mice had an expansile, multilobular mass composed of a chondroid cells exhibiting mild to moderate anisokaryosis, anisocytosis, numerous binucleate cells but no mitoses within the tibiotarsal joint that appeared to replace an area of the calcaneal tendon. Proliferative chondroid lesions, with or without evidence of endochondral ossification, also were present within the periosteum of the talus. Conclusion: The masses were interpreted as representing either a hyperplastic or a low-grade neoplastic process that appeared to arise from and replace areas of calcaneal tendon. The proliferations involving the periosteum of the talus were interpreted as chondroid metaplasia. Impact Statement: The etiology/pathogenesis of these unusual lesions is unclear; however, their prevalence (2/9 huNOG mice in this study) suggests potential implications for future studies involving the huNOG mouse model.
A Retrospective Study of Background Histologic Findings in New Zealand White Rabbits

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Introduction: New Zealand White rabbits are an important model system used in many toxicology studies; thus, discrimination between spontaneously occurring findings and compound-induced pathology is critical. Experimental design: The incidence of histologic findings in 296 control New Zealand White rabbits (129 male, 167 female) used in toxicologic studies at MPI between 2006-2014 was determined. Methods: Histologic findings were sorted by organ system, sex, and age group. Age groups were defined as <8 months (57 male, 49 female), 8-12 months (63 male, 89 female), and >12 months (9 male, 29 female). The percent incidence of each finding was calculated, and those with >1% incidence were considered notable. Findings were ranked according to incidence and compared between sexes and age groups. Results: Histologic findings were commonly (incidence >10%) seen in the thymus, kidneys, lung, spleen, reproductive organs, lymph nodes, and thyroid gland. Thymic lymphoid depletion (50%), kidney mineralization (37%) and tubular degeneration/regeneration (31%), inflammatory infiltrates in the liver (28%), and ovarian mineralization (20%) were the most common findings. There was no apparent sex or age predilection for any of the findings. Conclusion: There are a range of background findings that occur within untreated New Zealand White rabbits, and thus the relationship of these findings to test article should be carefully interpreted. Impact Statement: This comprehensive assessment of background findings in a large number of New Zealand White rabbits should improve future toxicology studies by allowing for greater discrimination between incidental and compound-induced changes.
Evaluation of Different and Alternative Fixatives in Substitution of Formalin in Toxicological Pathology

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Introduction: Pathological evaluation is a key element in toxicological studies. Formalin fixation is the predominant fixative in the anatomical pathology laboratory. However, the concern that formaldehyde can cause cancer induced governmental organizations to suggest restriction and even elimination of formaldehyde use. As a result of these considerations, in RTC pathology laboratory, different alternative fixatives to formalin were tested to investigate the morphological differences in organs/tissues sampling in toxicological studies.

Material and Methods: In 16 rats [Crl:CD (SD)] and 4 Göttingen minipigs, of both sexes, forty organs/tissues were fixed and tested in alternative fixatives as Greenfix, Excell Plus, and Fine Fix and Bouin’s 2000 fluid. After dehydration and embedding in paraffin wax, sections of all tissues were cut at 5 µm and stained with haematoxylin and eosin. The prepared slides were evaluated by different RTC pathologists for histological appearance and artifacts.

Results/Conclusion: The quality of rat or minipig tissue samples, following “RITA Trimming Guide”, fixed with the alternative fixatives, was found to be lower than formalin fixation, for the presence of numerous artifacts. We can therefore conclude that in the toxicological pathology, it is not possible to replace formalin fixative since the alternatives tested are not able to reach the same results and compromise the pathological evaluation.

Impact statement: Based upon our evaluation, the alternative fixatives available on the market are not able to substitute formalin as fixative in the toxicological pathology.

References

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Histology Provides Essential Context When Characterizing Chondrogenic Differentiation of Canine Bone-Marrow-Derived Mesenchymal Stromal Cells

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Introduction: Canine bone marrow-derived mesenchymal stromal cells (BM-MSCs) are a promising treatment option for repair of osteochondral defects. Unfortunately, inducing consistent cartilaginous differentiation with canine BM-MSCs is challenging. To further elucidate methods for consistent cartilaginous differentiation, the effects of dose and combination of three chondrogenic growth factors were assessed in a three-dimensional (3D) collagen type I scaffold using histology, proteoglycan production, and real-time PCR for chondrogenic and osteogenic genes.

Methods: Canine BM-MSCs were isolated, characterized, and placed in a 3D collagen type I scaffold with basal chondrogenic differentiation medium. Cultures were supplemented with transforming growth factor beta-3 (TGF- b3), and varying doses of bone morphogenetic protein 2 (BMP-2) and/or basic fibroblast growth factor (bFGF). Chondrogenic and osteochondral differentiation were assessed via quantification of typical chondrocyte products (glycosaminoglycans) and quantitative real-time PCR for Sox 9, collagen type II, collagen type I, aggrecan, osteocalcin, and osterix. Additionally, histology with routine hematoxylin and eosin staining, as well as staining with toluidine blue and safranin O was performed.

Results: Synthetic and genetic assays supported successful chondrogenic differentiation of canine BM-MSCs when combined with certain growth factor combinations and doses. However, histology revealed heterogeneous spindle cells that did not exhibit typical articular cartilage morphology despite some cellular staining with toluidine blue and safranin O.

Conclusion: The discrepancy between histology results and other metrics highlights the importance of histology when evaluating chondrogenic differentiation. Regardless of transcriptional and matrix deposition changes, histology should be considered an essential assessment tool for chondrogenic differentiation studies.

Impact statement: Histology provides essential context regarding the degree of chondrogenic differentiation of canine BM-MSCs.
Toxicity of Hydroxyurea in Cynomolgus Monkeys

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Introduction: Hydroxyurea (HU) is used to treat a number of conditions, including Sickle Cell Disease, melanoma, and myelocytic leukemia. Detailed toxicity data of HU in monkeys is lacking in the literature.

Experimental Design: To investigate HU toxicity in the cynomolgus monkey, 21-day escalating dose tolerability and 3 month toxicity studies were conducted. Methods: Cynomolgus monkeys received daily oral administration of 40, 80, or 160 mg HU/kg/day in the 21-day study and 60 mg/kg/day in the 3-month study. Endpoints for both studies included: clinical observations, body weights, hematology, coagulation parameters, serum chemistry, and toxicokinetics; additional endpoints for the 3-month study included: ophthalmology, urinalysis, leukocyte immunophenotyping, organ weights, and gross and histopathology.

Results: HU administration in the 21-day study resulted in time- and dose-dependent decreases in RBC, HGB, HCT, WBC, reticulocytes, neutrophils, monocytes, eosinophils, and platelets, and increases in RDW and HDW at 80 and 160 mg/kg/day. At 60 mg/kg/day in the 3-month study, several animals showed signs of increased inappetence, and decreases in RBC, HGB, HCT, and increases in MCV, RDW, MCH, and HDW (Cmax: 22.0 µg/mL; AUC: 164 µg·hr/mL). No effects were observed in the bone marrow by histology or in the leukocyte populations by immunophenotyping. Small thymus and lower thymus weights observed at necropsy correlated histologically with increased incidence/severity of decreased thymic lymphocytes.

Conclusions: HU toxicity in the cynomolgus monkey was characterized by inappetence and decreases in erythrocytes and, at higher doses, leukocytes.

Impact Statement: These studies provide valuable HU safety data in cynomolgus monkeys to the literature.
"Adversity of Lysosomal Accumulation" in Toxicity Studies, The Pathologists' Point of View—Results from the 5th ESTP International Expert Workshop in Barcelona, Sept. 23–24, 2016

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The European Society of Toxicologic Pathology (ESTP) organized a series of international workshops due to the recognized need of the toxicological community to better align on the determination of nonclinical adversity. An introductory workshop in 2015 defined and characterized adversity and developed recommendations for a practical approach to evaluate adversity in toxicity studies (Palazzi et al., 2016).

Building on this, a lesion-specific workshop entitled “Adversity of Lysosomal Accumulation”, was held in Barcelona, Spain on September 23–24, 2016. Lysosomal accumulation is a common issue in nonclinical studies, as a result of the specific role of lysosomes in cellular uptake, degradation and health, and because it is associated with the physicochemical properties of certain pharmaceutical and chemical agents.

Twenty-three international expert pathologists, toxicologists and research scientists representing pharmaceutical and chemical industries, contract research organizations and regulatory authorities from Europe, the United States, and Japan met in Barcelona, after a 7-month preparatory phase of teleconferences with initial expert contributions. The approach of this workshop was holistic taking into account other analytical and research methods available for assessing lysosomal accumulation, beyond the routine H&E staining.

This poster summarizes workshop discussions, examples of lysosomal storage and practical considerations to assist study pathologists in the adversity assessment. In alignment with the general recommendations made by Palazzi et al., 2016, the following key factors are proposed for the adversity assessment of lysosomal accumulation: 1) The anatomical location and cell population affected, 2) The nature of accumulated material (endogenous versus exogenous), 3) Functional consequences, and 4) Severity.
Intertubular Seminoma in Sprague-Dawley Rats: A Novel Presentation of a Rare Rodent Tumor?

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Seminomas are rarely reported in rodent species. Most documented cases exhibit invasion or features of malignancy. However, a number of well-differentiated, intratubular seminomas, closely resembling the spermatocytic seminoma variant described in humans, have been reported in Sprague-Dawley rats. Here, we present several rat seminomas in Sprague-Dawley rats from a 2-year carcinogenicity study with perinatal exposure. These tumors were diagnosed by a pathology working group (PWG) as "interstitial" or "intertubular" seminoma, characterized by well-demarcated aggregates of neoplastic germ-like cells confined to the interstitial spaces between seemingly unremarkable seminiferous tubules. Given that this seminoma variant has not been previously described, immunohistochemistry techniques were utilized to confirm the diagnosis and determine if there was differential staining between seminoma variant types. In this study, three, "intertubular" seminomas from the PWG, and four, seminomas from the NCTR archives were collected for light microscopic and immunohistochemical evaluation. All seminomas were stained with a variety of antibodies including c-kit, CD45, PLAP and CD30 to help confirm the diagnosis of "intertubular" seminoma- a new variant of seminoma in Sprague-Dawley rats. While the seminoma remains a rare tumor in rodent species, we may see more of the "intertubular" variant in future developmental exposure studies.
Using the Chemical Effects in Biological Systems (CEBS) Database to Answer Pathology Questions

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Introduction: The Chemical Effects in Biological Systems database (CEBS: https://tools.niehs.nih.gov/cebs3/ui/) hosts many data types, including a large repository of pathology data from over 650 test articles in the NTP toxicological testing program. These data can be accessed using CEBS guided searches with a variety of search criteria. Our objective was to use CEBS to evaluate two questions. First, does route of administration affect liver tumor incidence in male B6C3F1 control mice? Second, are mammary tumors related to pituitary tumors in female F344 rats?

Methods and Materials: Using the CEBS NTP Pathology guided search and Oracle SQL queries for supporting information, we determined the incidence of tumors in male B6C3F1 control mice and in female F344 treated and control rats from chronic (2-year) studies. Results: The percent of male mice with hepatocellular tumors was significantly different between routes of exposure based on analysis of variance (P<0.001), where control groups of oral feed had the lowest incidence (38%) and control groups of oral water (72%) and topical application (65%) had the highest incidences. In addition, chi-square analysis demonstrated that female rats with pituitary tumors were more likely to have mammary tumors than rats with no pituitary tumors (P<0.001).

Conclusions: Pathology data in CEBS can be mined to answer data-driven questions. These data can be downloaded and opened in Excel for further analysis, including statistics. We showed that incidence rates of liver tumors in control mice might be related to exposure route, and a possible association between rat mammary and pituitary tumors.
Characterization of Focal Ectopic Myelinated Nerve Fibers in the Retina of a Cynomolgus Monkey: A Spontaneous Background Finding

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Introduction: Myelinated retinal nerve fiber layers (MRNFL) are lesions of abnormal myelination of the retinal nerve fibers. They appear ophthalmoscopically as white patches on the retinal surface. While ophthalmologic characterization of the MRNFL has been well documented, minimal information is available concerning microscopic description in nonhuman primates. We report herein the histopathologic features of a spontaneous case of MRNFL. Experimental Design: During the routine ophthalmic examinations of cynomolgus monkeys used in a regulatory toxicology study, unilateral MRNFL was detected in a 4-year old monkey. The lesion was seen as a solitary focus in the central area of the fundus which involved the optic disc, proceeding and at the end of the study, indicating a pre-existing lesion. Eyes were submitted for microscopic examination. Methods: Tissues were processed in a routine manner for histologic evaluation and stained with hematoxylin-eosin and luxol fast blue. Tissues were also immunohistochemically stained with mouse monoclonal antibody specific for 2',3'-cyclic nucleotide 3'phosphodiesterase (CNPase). Results: Microscopic examination revealed a well demarcated, non-encapsulated mass composed of a uniform population of spindle cells arranged in fascicles at the optic disc. The cells had uniform round-to-oval nuclei and eosinophilic fibrillar cytoplasm with indistinct cell borders. There was no identifiable mitotic activity. The cells were intensely positive for luxol fast blue and moderately positive for CNPase. Conclusion: The MRNFL was considered to be due to the presence of ectopic oligodendrocyte-like cells in the retina. Impact Statement: This report provides the microscopic characterization of an unusual spontaneous background retinal lesion in a monkey.
Exacerbation of Seminiferous Tubule Dilatation by Fibrous Hypoplasia in Cynomolgus Monkey Testes

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Introduction: Incidental findings in the testes of Cynomolgus monkeys include tubular luminal dilatation and replacement of seminiferous tubules by excessive collagen (fibrous hypoplasia). This study reviews the background incidence and severity of seminiferous tubule dilatation and fibrous hypoplasia in the testes of Asian Cynomolgus monkeys and the potential exacerbation of the tubule dilatation by the fibrous hypoplasia.

Methods: A retrospective survey was conducted in 212 purpose bred Asian Cynomolgus monkeys, used in safety assessment studies conducted between 2009 and 2011. H&E stained testes were re-examined microscopically. The incidence of tubular dilatation was evaluated with respect to sexual maturation (using testicular morphologic criteria), the presence of fibrous hypoplasia, and distribution.

Results: A total of 21/212 (10%) monkeys had focal or segmental tubular lumen dilatation. Tubule dilatation was not observed in immature monkeys. Minimal to moderate tubule dilatation was observed in maturing and mature monkeys, involving tubules close to the rete and/or capsule and occurred unilaterally (57%) or bilaterally (43%). Fibrous hypoplasia was present in 53/212 monkeys (25%). Fibrous hypoplasia was observed in immature, maturing and mature monkeys. All mature monkeys with fibrous hypoplasia also had tubular dilatation of predominantly moderate severity and associated with pressure atrophy of the seminiferous epithelium.

Conclusion: The presence of fibrous hypoplasia in the testes of sexually mature Cynomolgus monkeys appears to exacerbate the incidence and severity of tubular dilatation, resulting in pressure atrophy of the dilated tubules. It is important that these background lesions are distinguished from test article related changes.
Lipopolysaccharide-Mediated Autophagy Protects against Thioacetamide-Induced Acute Liver Injury in Rats

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<Introduction> Autophagy is a critical lysosomal pathway that maintains cellular homeostasis and survival. The current research was to assess the role of autophagy in thioacetamide (TAA)-induced acute liver injury and determine its potential pathophysiological relevance. <M&M> 6-week old F344 male rats were injected with TAA (100 mg/kg, i.p.) and liver samples were collected at post injection 0 h to 5 d. For autophagy stimulation, lipopolysaccharide (LPS; 0.1 mg/kg, i.p.) was treated 2 h before TAA injection. <Results> AST value was significantly decreased in autophagy-activated rats (LPS+TAA) compared with Saline+TAA rats on 1 d. TAA induced coagulation necrosis of hepatocytes on 1 to 3 d and subsequent reparative fibrosis on 5 d in the centrilocular areas. Interestingly, liver injury was reduced in LPS+TAA rats in contrast to Saline+TAA rats. LC3B (autophagy marker) expression was sharply increased in both groups on 1 to 3 d and localized around the necrotic areas; however LC3B-labeled dot number within the hepatocyte was significantly increased in LPS+TAA rats. LPS-induced autophagy resulted in less hepatocyte cell death and increased proliferative activity of hepatocytes in LPS+TAA rats. Fibrogenesis-related genes (collagen 1α, TIMP2, MMP2) were significantly decreased in LPS+TAA rats compared with Saline+TAA rats on 3 d. <Conclusions> LPS-induced autophagy defends against hepatocellular death, enhancing hepatocyte proliferative activity and reducing fibrogenic factors in TAA acute liver injury. Further investigations on autophagic signaling for hepatocyte apoptosis and proliferative activity are underway.
A Comparison of Autoimmunity Induced by Nivolumab or Ipilimumab in Two Humanized Mouse Models

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Introduction: Immune checkpoint inhibitors used to enhance the immune system have demonstrated remarkable clinical effectiveness in the treatment of cancer. Primary targets for inhibition include programmed cell death-1 (PD-1) and cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4). Despite important clinical benefits, some patients have experienced a spectrum of immune-related, adverse events (irAEs) that non-clinical, non-human primate studies failed to predict. Experimental Design: Herein, the bone marrow-liver-thymus (BLT) immune humanized mouse model was used to determine predictability of irAEs. Nivolumab or ipilimumab, anti-PD-1 and CTLA-4 inhibitors respectively, were used to study immune-mediated effects in NOD.Cg-Prkdcscid Il2rgtm1Sug/JicTac (NOG) and in the NOG/hGM-CSF/hIL-3 BLT humanized mice. Methods: Mice were treated with 2.5, 5.0, or 10.0 mg/kg nivolumab or ipilimumab or saline intraperitoneally twice weekly for four weeks. Mouse body weights were monitored twice weekly and observations of behavior and appearance were noted daily. Bloodwork was completed pre-study, at 14 days, and necropsy. Necropsy was completed at 28 days or sooner if weight loss >20% or morbidity was noted. Results: Similar to irAEs reported in humans, histopathologic findings in nivolumab and ipilimumab treated mice showed that mice across all dose groups developed variable severities of pneumonitis and hepatitis, with nephritis, dermatitis, and colitis noted in some animals. Additional findings included pancreatitis, pancreatic atrophy, adrenalitis, myositis and osteomyelitis. Flow cytometric analysis showed increased T-cell activation with decreased CTLA-4 and loss of PD-1 expression. Treatment effect was strain dependent. Impact statement: These findings suggest that this humanized mouse model can demonstrate irAEs similar to those observed in humans.
Measuring Thrombin Generation in Rats Using the Calibrated Automated Thrombogram

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Objectives: To measure thrombin generation (TG) using the Calibrated Automated Thrombogram (CAT®, Thrombinoscope BV, Stago, France) assay in healthy rats citrated poor platelet plasma (PPP), to assess precision and sensitivity to spiked tissue factor (TF) and unfractionated heparin (UFH). Methods: TG was measured according to the manufacturer's method. Clotting was initiated using a trigger solution containing phospholipids (4μM) and tissue factor (5pM) (PPP-Reagent®, Stago, France). Endogenous thrombin potential (ETP), lag time, time to peak, peak and start to tail were recorded. Experimental Design: PPP from 50 healthy male and female rats was kept at -80°C prior to analysis. Intra and inter-assay precisions were assessed on pooled plasma from 5 male and 5 female rats. Inter-individual variability was evaluated on 10 male and 10 female rats. Pooled plasma samples were spiked with increasing concentrations of UFH (Heparin, Sandoz®) or recombinant human TF (rh-TF, Innovin®). Results: Intra-assay coefficient of variation (CV) was ≤ 5%, inter-assay ≤ 20 %, inter-individual CV ≤ 30 %. Compared to baseline, heparin decreased TG in a dose-dependent manner with decreased ETP and peak. Similarly, rh-TF accelerated TG with decreased lag time, time to peak, start tail and increased peak concentration of thrombin. Conclusions: The CAT assay can be used to measure TG in rats with good precision. This assay is sensitive to the ex vivo effect of rh-TF and heparin. Impact statement: In preclinical studies, the CAT assay could be used to detect drug-induced hyper and hypocoagulability in rats.
Selection of a Novel Lipid Nano Particles (LNP) Vehicle for Intramuscular Injection of Therapeutic mRNA

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Delivery of nucleic acids as a therapeutic intervention for disease encompasses various approaches from virus mediated DNA transduction to delivery of DNA and RNA in micellar particles such as liposomes. We have focused on the delivery of mRNA using lipid nanoparticles (LNPs) by parenteral routes of administration (ROAs). Of particular interest to our ventures are the intramuscular (IM) and subcutaneous (SC) ROAs. Unfortunately, these methods of mRNA/LNP delivery are often associated with an inflammatory injection site reaction (ISR) characterized by a dose-limiting innate immune response leading to injection site swelling, infiltration of neutrophils and macrophages and destruction of surrounding tissue. ISRs in preclinical species are highly translatable to humans and, depending upon severity, might be an unacceptable adverse event during clinical trials. Here we compared five novel LNPs to an ISR positive control in a single-dose *in vivo* rat model for ISR. These lipids were injected IM in Sprague Dawley rats at 10 or 100μg in 100μL and ISR was assessed by histopathology and cytokine panel. The cytokine panel showed some correlation with the severity of ISR. Histopathology of the skin and muscle at the site of injection showed varying degrees of inflammatory cell infiltrate, neutrophil degeneration and muscle fiber necrosis. These reactions were graded by blind assessment which enabled rank ordering LNPs for ISR severity and aided the selection of an IM delivery vehicle.
Development of Digital Tissue Image Analysis Solution for Muscle Biopsies in Support of Disease-Modifying Therapies for Duchenne Muscular Dystrophy

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Introduction: The continual expression of utrophin protein by pharmacological maintenance of utrophin transcription in dystrophin-deficient muscle fibers is potentially a disease-modifying treatment for Duchenne muscular dystrophy (DMD) regardless of the dystrophin mutation. The evaluation of molecular biomarkers of muscle regeneration, such as developmental myosin, may be important endpoints in future clinical trials of utrophin modulators. Building on the recently published manual quantification approach which demonstrated a positive correlation between utrophin levels and the degree of muscle fiber regeneration in DMD and Becker muscular dystrophy (BMD) muscle biopsies, the development of fully automated processes has now been completed. Methods and Materials: Here, we report the development of multiplex immunohistochemical (IHC) assays and computational tissue analysis (cTATM) solutions to robustly quantify utrophin and developmental myosin heavy chain expression in acetone-fixed, frozen DMD, BMD, and control muscle biopsy sections obtained from the Paul D. Wellstone Muscular Dystrophy Cooperative Research Center. The cTA approach enabled detection of biomarker signal features (e.g., cumulative intensities) and tissue morphometrics (e.g., fiber area and minimum diameter) of individual muscle fibers in whole-slide images of muscle cryosections. Results: The cTA solutions reproducibly demonstrated quantifiable differences in levels of utrophin, and regeneration between DMD, BMD, and control biopsies. Conclusion: These biomarkers may be informative endpoints for evaluating pharmacologic benefit in dystrophin-deficient muscle in future clinical trials of utrophin modulators and potentially other DMD therapeutic approaches.
Comparison of Survival, Tumor Incidence, and the Incidence of Chronic Progressive Nephropathy in Carcinogenicity Studies Conducted in CD®IGS Rats and CD-1®IGS Mice Fed 5002 or 5CR4 Certified Diets

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LabDiet certified rodent 5002 and 5CR4 diets are two diets used in GLP carcinogenicity studies. One of the major differences between the two diets is the protein content (5002 contains ~20% protein; 5CR4 contains ~14% protein). Experimental design: To assess possible differences in the effects of these diets on carcinogenicity study endpoints, historical control parameters from carcinogenicity studies conducted within the last 5 years using 5CR4 diet and within the last 10 years using 5002 diets at our facility were compared in male and female CD®IGS rats (Crl: CD(SD)) and CD-1®IGS (Crl: CD1(ICR)) mice.

Methods: Among the parameters evaluated were survival, tumor incidence, and the incidence of chronic progressive nephropathy (CPN) in rats and mice. Results: Tumor profiles were generally comparable between animals fed 5CR4 or 5002 diets. The 5CR4 diet was associated with greater survival in male rats. The 5CR4 diet was associated with a lower incidence of chronic progressive nephropathy in male and female rats and mice. Conclusion: Further investigation is needed to determine the influence of the two diets on the severity of nephropathy and the role of nephropathy in survival. Impact statement: Based upon our initial evaluation, 5CR4 which has a lower protein content increases the survival in male rats and lowers the incidence of chronic progressive nephropathy in male and female rats and mice.
Constitutive Androstane Receptor (CAR)-Dependent Liver Tumor Induction by Imazalil in Mice

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Introduction: Imazalil (IMA), an imidazole fungicide, increased an incidence of hepatocellular adenoma in mice through non-genotoxic mechanism. IMA was also inducible liver hypertrophy and cytochrome P450 (CYP) activity. However, no further mechanistic analysis of the hepatocarcinogenesis or liver hypertrophy has been conducted.

Objective: To clarify a major pathway of liver tumor development by IMA, we focused on CAR, a key nuclear receptor for mouse liver tumorigenesis. We also examined involvement of other nuclear receptors in liver hypertrophy induced by IMA.

Materials and Methods: Male CAR-knockout (CARKO) and wild-type mice were dietary treated with IMA at 500 ppm for 27 weeks after initiation by diethylnitrosamine. For dose dependent analysis of hepatic drug metabolite enzymes induction, multiple doses of IMA were administered to mice for a 1-week period.

Results: At the termination, IMA treatment statistically significantly did not increase altered foci or adenomas in the liver of CARKO mice compared to those in controls, whereas the treatment increased both eosinophilic altered foci and adenomas in wild-type mice. For 1-week of IMA treatment, liver hypertrophy was induced in both genotypes without differences among genotypes. Analysis of CYPs expressions indicated that pregnane X receptor was involved in liver hypertrophy based on markedly elevation of Cyp3a11 and Cyp2b10 expression levels in a dose-dependent manner in both genotypes by IMA.

Conclusions: Our results demonstrated that the CAR mediated pathway was the main mechanism of liver tumor development induced by IMA.
Alterations in MAPK Signaling in Alveolar Bronchiolar Carcinomas from B6C3F1/N Mice Exposed to Antimony Trioxide by Inhalation for Two Years

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Introduction: Antimony trioxide (AT) is used as a flame retardant in textiles, paper, and plastics. Occupational exposure to antimony is a concern in the metal ore smelting and mining industries. Recent NTP studies show that chronic exposure of B6C3F1/N mice and Wistar Han rats to AT particulates for 2 years resulted in increased incidences of alveolar bronchiolar tumors (ABC). Experimental Design: Mutation analysis (rats and mice) and transcriptomic analysis (mice only) were performed on ABCs resulting from AT-exposure. Methods: DNA isolated from formalin-fixed, paraffin-embedded tissues was PCR amplified and subjected to Sanger sequencing to identify mutations in Egfr and Kras genes. RNA isolated from frozen tissues was used for Genechip arrays. Results: Incidences of Kras and Egfr mutations in ABC from mice exposed to AT were 43% (34/80) and 46% (37/80), respectively. ABCs from rats also harbored mutations in Egfr (50%, 13/26) but not in Kras (1 non hotspot mutation). Transcriptomic analysis demonstrated alterations in eukaryotic initiating factor-2 signaling (EIF2), and regulation of EIF4 and p70S6K signaling. Conclusion: Both Kras and Egfr are major components of the MAPK signaling pathway but AT-induced lung tumors seem to preferentially harbor Egfr mutations in both mice and rats. Collectively, the Egfr mutations in dual species and the alterations in EIF2 and IEF4/p70S6K signaling pathways in mouse ABCs suggest MAPK signaling is important in AT-induced pulmonary carcinogenesis. Impact statement: Alterations in MAPK signaling in AT-induced rodent lung tumors may be relevant in the context of human lung cancers.
Exome Sequencing of Spontaneous and Chemically Induced Hepatocellular Carcinomas in B6C3F1/N Mice Identifies Unique Somatic Mutations

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Introduction: Hepatocellular carcinoma (HCC) is the third-leading cause of cancer-related death in the world. The B6C3F1/N mouse has a moderate background incidence of HCC and increased incidences due to chemical exposures are frequently observed in NTP bioassays. A better translational insight into the mouse HCCs can be gained from a comprehensive evaluation of mutations in HCCs that arise either spontaneously or due to chemical exposures.

Experimental Design and Methods: Whole exome sequencing was performed on DNA extracted from fresh-frozen liver tissues of B6C3F1/N mouse HCCs arising either spontaneously (n=3) or due to chronic exposure to genotoxic carcinogens such as Gingko Biloba Extract (GBE) (n=3), and Methyl Eugenol (MEG) (n=3) as well as age-matched normal livers (n=3). The genomic sequence data from the parental strains C57Bl/6 and C3H and mm9 were used as the reference genome for variant calling.

Results: Spontaneous tumors harbored mutations in Hras (Q61K); GBE-induced HCC harbored mutations in Ctnnb1(T41A) and MEG-induced tumors harbored mutations in Braf (V637E), Hras (Q61R), and Ctnnb1 (D32N, D32Y). Mutations in Bves (R180S, P195L) were noted in HCCs from all three groups. Mutational signature analysis are in progress.

Conclusion: Unique risk-factor-specific genetic alterations were identified in spontaneous and chemically induced mouse HCC.

Impact statement: Etiology-dependent unique somatic mutations in mouse HCCs have emerged as potential biomarkers of exposure and neoplasia. Targeted sequencing analysis of tissues from subchronic studies have the potential to predict carcinogenicity and/or chemical exposures. Additional data from larger sample sizes will provide greater confidence in this data and approach.
Intravitreal D-Arginine Administration Results in Retinal Degeneration and Necrosis in Male New Zealand White Rabbits

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Introduction: High viscosity is a common problem in the development of therapeutics for intravitreal administration. Excipients may be added to drug substance to reduce viscosity, however many traditional excipients are not suitable for intravitreal (ITV) administration. This study aimed to characterize the tolerability and toxicity of D-arginine as a novel excipient for intravitreal administration in the rabbit. Methods: Rabbits received a single ITV dose of buffered D-arginine- HCl solution ranging from 3.5 to 8.7mg/eye (375-816 mOsm/kg), or an osmolality-matched vehicle. Indirect ophthalmoscopy and slit lamp biomicroscopy were used to evaluate effects of treatment on the eye during the in life phase of the study. Animals were necropsied on day 2 (interim) or 8 (terminal), and the eyes were submitted for histopathological examination. Results: ITV administration of D-arginine resulted in retinal degeneration and necrosis at doses of 3.5 mg/eye and higher. High tonicity vehicles alone induced retinal changes at 534 mOsm/kg and above, comprising retinal detachment, vacuolation of the photoreceptor outer segments and atrophy of the outer nuclear layer. Conclusions: These data show that D-arginine is not a suitable excipient for ITV drug administration in rabbits. Further work is required to determine the mechanism of toxicity.
Retinal Toxicity Associated with Intravitreal Administration of Hyper- and Hypo-osmolar Vehicles

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Introduction: Little is known about the safety of intravitreal (ITV) ophthalmic formulations outside of isotonic ranges, yet hyperosmolar formulations are increasingly being brought forward for development. This work was undertaken to assess the impact of the intravitreal administration of high and low osmolality solutions on the eye. Methods: Rabbits were randomized to 8 groups (n=3 animals), receiving hypotonic, isotonic or variably formulated hypertonic solutions via two 50 µl intravitreal injections, 15 minutes apart. In addition, we investigated the effects of ziv-aflibercept (ZALTRAP®), an anti-VEGF used off-label for wet age-related macular degeneration (wAMD), which is a hypertonic formulation. Animals were monitored for 8 days via ophthalmic examination (OE) and Optical Coherence Tomography (OCT). On Day 8 animals were euthanized and the eyes were processed for histopathologic examination. Results: Sporadic focal subretinal opacifications were noted at OE in hypotonic and elevated NaCl hypertonic formulations. Inferior retinal detachment was seen via OCT following ITV administration on Day 1 in a proportion of animals treated hyperosmolar solutions, including ziv-aflibercept, but this had resolved by Day 3. Microscopically, hypotonic or hypertonic formulations, including ziv-aflibercept, administered ITV to rabbits resulted in focal to multifocal posterior retinal degeneration of minimal to marked severity, which was most severe in animals receiving the hypotonic formulation. Conclusions: These data indicate hypo- and hyperosmolar solutions may themselves be retinotoxic, and formulations of 71mOsm/kg or 849-1063 mOsm/kg may confound the safety evaluation of ITV therapeutics in rabbits.
Submandibular Blood Collection in C57Bl/6 Mice Associated with Hemorrhagic and Necrotizing Brain Lesions

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Introduction: Submandibular blood collection is an alternative, rapid, simple, and humane method for drawing blood samples from mice. In the literature, it has been associated with local tissue injury at the puncture site and occasionally accidental punctures into the ear canal or oral cavity, but is otherwise considered minimally injurious. Experimental Design: In a 1-year toxicity study, male C57Bl/6 mice (n=20/group) were administered either vehicle or test article and underwent at least 18 submandibular blood collections. In a 10-day pilot study, untreated male C57Bl/6N mice (n=3-4/group) underwent 1 or 3 submandibular blood collections during a 2- or 10-day period, respectively, prior to necropsy. Methods: Brain (toxicity study) or whole head with brain (pilot) was formalin-fixed, sectioned transversely and examined microscopically. Results: In the 1-year study, repeated submandibular blood collection was associated with localized areas of hemorrhage, necrosis and parenchymal loss with gliosis, hemosiderin deposition and occasionally intravascular/perivascular hair shafts. Brain findings were generally unilateral, ventrolateral and localized to the hypothalamus, thalamus, midbrain, pons, hippocampus or ventrolateral cortex. In the 10-day study, submandibular collection was associated with inflammation and hemorrhage at the puncture site extending to the brain through an opening in the skull, the petrosquamosal fissure. Conclusion: Repeated submandibular blood collections in mice were associated with hemorrhagic and necrotizing brain lesions in C57Bl/6 mice, which were attributed to vascular compromise and/or direct extension of procedure-related tissue injury. Impact: In future studies using this blood collection technique, similar microscopic findings in the brain may be directly attributed to this technique.
Radiation-Induced Renal Changes in the Göttingen Minipig

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Introduction/Objectives: During the development of a hemi-body shielded model of radiation-induced gastrointestinal injury (GI-ARS) in Göttingen minipigs, unique morphologic effects were identified within the kidneys, including necrosis and regeneration of the collecting tubules, which have not been previously described.

Experimental Design: Male and female Göttingen minipigs were hemi-body shielded and exposed to a single uniform total body dose of gamma irradiation at dose levels of 14 to 16 Gy. Animals were necropsied at several intervals ranging from Study Day 10 to 60.

Methods: At the time of necropsy, tissues placed into 10% NBF, routinely processed and embedded in paraffin, sectioned, and stained with H&E. Tissues from selected animals were evaluated by light microscopy.

Results: As early as Study Day 14, necrosis and regeneration were observed within the collecting tubules of the medulla and the medullary rays in the kidney. The change was most severe in animals necropsied at Study Day 30 and later. Other changes more consistent with radiation toxicity were also observed in the kidney.

Conclusion: The Göttingen minipig appears to have a unique morphologic response to high doses of radiation exposure in the kidney following sufficient hemi-body shielding to spare the hematopoietic system.

Impact Statement: The effects of high doses of radiation exposure on the kidney of Göttingen minipigs are discussed. A unique feature of necrosis and regeneration of the collecting tubules was observed. This may represent a species-specific effect that other scientists should be aware of when working with this GI-ARS model.
Comparison of the Intramuscular Tolerability to Five Long-Acting Drug Nanosuspensions with Similar Particle Sizes but Different Stabilizing Excipients, following a Single Intramuscular Administration in the Rat

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The purpose of this study was to investigate the influence that common nanosuspension-stabilizing excipients have on the nature and temporal evolution of the histopathological/immunological changes at the intramuscular (i.m.) injection site and draining lymph nodes, following i.m. administration. Each of 5 groups of 39 male rats received a single injection of one of the analogous crystalline drug nanosuspensions containing 200 mg/mL of an anti-viral compound with particle sizes of ±200 nm and identical vehicle compositions, except for the type of the nanosuspension stabilizer. The stabilizers included were: poloxamer 338, poloxamer 407, D-α-tocopherol polyethylene glycol 1000-succinate (TPGS), polysorbate 80, and polysorbate 80 combined with egg-phosphatidylglycerol. Histopathology and immunohistochemistry findings revealed varying degrees of inflammation at the administration sites and draining lymph nodes that differed according to the time post-dose and the type of stabilizer. Overall, the progression of the inflammatory changes was similar across the groups, but differences in the nature and severity of the histopathology findings were observed between animals injected with poloxamer-/TPGS-containing nanosuspensions and those injected with polysorbate-containing nanosuspensions. More severe inflammatory changes, including the presence of multinucleated giant cells, prolonged histiocytic infiltrations of the formulation depots, and a slightly prolonged persistence of histiocytic infiltrates in the lymph nodes, were observed in animals injected with polysorbate-containing nanosuspensions. Such vehicle-mediated effects could influence the overall tolerability profile of long-acting nanosuspensions.
Spontaneous, Treatment-Related, and Iatrogenic Lesions in the Nasopharyngeal Ducts of Rats and Mice

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Introduction: Nasal cavity lesions in rats and mice are well-documented. However, there are few reports in the literature that specifically address nasopharyngeal duct (NPD) lesions. Spontaneous proliferative lesions in rat nasal passages and submucosal neutrophil infiltration associated with a foreign body have been described. Inhalation-induced NPD lesions have occurred in rats and/or mice in a few 90-day studies conducted by the National Toxicology Program (NTP). Experimental Design: To determine the occurrence of NPD lesions in chronic studies, we searched the NTP database of 450 studies in rats and mice from untreated control, vehicle control, and treated groups. Methods and Materials: The findings were sorted and characterized as spontaneous, iatrogenic, or treatment-related according to the incidences in control animals compared to those in treated groups. Results: Nasopharyngeal duct lesions, principally inflammation, foreign body, and respiratory epithelial hyperplasia or degeneration, were recorded in control and treated rats and/or mice from 28 chronic studies. The highest incidences of NPD lesions occurred in rats and mice exposed by gavage to Green Tea Extract and in mice exposed by inhalation to the Metal Working Fluid, Trim VX. Conclusion: Nasopharyngeal duct lesions in rats and mice exposed to Green Tea Extract were considered a sequela of gavage-related reflux; those associated with Trim VX were considered treatment-related. All other occurrences were considered spontaneous. Impact statement: Understanding the occurrence and morphology of NPD lesions could aid in determining the pathogenesis of the lesions and thus interpretation of the studies.
Common Incidental and Procedural-Related Otic Microscopic Findings in the Guinea Pigs Used in Nonclinical Auditory Safety Studies

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Introduction: Guinea pigs are an important animal model used in auditory safety toxicology studies. This review highlights some common incidental microscopic observations ("background findings") in the naïve ear of this species and will discuss common procedural-related microscopic findings observed in the ear secondary to trans-tympanic and middle ear catheter dosing. Experimental design: The incidence of histologic lesions in guinea pig naïve ears and ears treated with saline or vehicle by transtympanic injection or middle ear catheter in ototoxicity studies at MPI between 2012-2017 was determined. Methods: Histologic lesions were graded and were sorted by otic compartment. Lesions of were ranked from highest to lowest incidence, and the percent incidence of each lesion was compared between naïve ears, ears treated with either saline or vehicle by transtympanic injection or middle ear catheter. Results: The most common histologic lesions in the naïve ear included new bone formation of the tympanic bulla and foamy macrophages of the tympanic cavity. Common histologic findings in ears treated with saline and/or vehicle by transtympanic injection included: inflammation, hemorrhage, and foamy macrophages in the tympanic cavity; and fibroplasia and inflammation in the tympanic membrane. Conclusion: These lesions appear to occur in naïve ears and/or are procedural related and should not be interpreted as occurring due to compound administration in auditory safety studies. Impact Statement: Knowledge of background lesions in the naïve and control (saline or vehicle) treated guinea pig ears should improve auditory safety studies by allowing for greater discrimination between incidental and compound-induced lesions.
Previously Diagnosed Reticulum Cell Lesions of Rat Bone Marrow Stain Positive for Ionized Calcium Binding Adapter Molecule 1 (Iba1)—a Monocytic/Macrophage Cell Marker

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Introduction: Reticulum cell hyperplasia (RCH) was a term used for many years by the National Toxicology Program (NTP) to describe a certain non-neoplastic bone marrow lesion of rats. Diagnosis of this lesion indicated the presence of focal to broadly diffuse areas of large cells characterized by an abundant cytoplasm that may contain vacuoles or appeared foamy with irregular, indistinct cell borders and a round to bean-shaped nucleus. Retrospective microscopic evaluation of RCH lesions and immunohistochemistry were performed to reassess and further characterize these cells. **Experimental Design/Methods:** Search of the NTP database to identify femoral bone marrow specimens diagnosed with RHC from 1981 to present time. Sixty-nine RCH slides, spanning 22 years, were selected for review, and a subset (18) chosen for immunohistochemical characterization of the cells. Initial investigations revealed ionized calcium binding adapter molecule 1 (Iba1) as the only macrophage marker that consistently worked on decalcified tissue; 3µm thick sections were stained with Iba1 antibody. **Results:** A total of 254 bone marrow specimens were diagnosed with RCH in the database. The diagnosis of RCH last occurred in 2003, after which the term “cellular infiltration” was used. The selected sixty-nine slides encompassed 29 chemicals. Upon reassessment, 26 were consistent with histiocytic cellular infiltration, 18 with histiocytic sarcoma, 19 with other diagnoses, and 6 were normal in appearance. 17/18 RCH lesions stained positive for Iba1. **Conclusion:** Lesions previously diagnosed as RCH are consistent morphologically and immunohistochemically with cells of histiocytic origin. **Impact Statement:** These results will help with interpretation of historical data.
Histological Methods for Laryngeal Function Studies in the Rat and Rabbit

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Introduction: The anatomic and histologic features of rats and rabbits have been described previously with a focus on inhalation toxicity and the changes that result in the upper respiratory system. Laryngeal pathology as it relates to voice disorders requires a change in focus from the highly sensitive areas examined in toxicology studies to the anatomical landmarks of phonation.

Methods: Rat and rabbit larynges were routinely fixed and embedded in paraffin. Sections were obtained in the coronal plane by microtoming from the dorsal/posterior surface of the larynx including the epiglottis to caudal portion of the cricoid cartilage. Cross-sections of the larynges were cut transversely through the cricothyroid indentation with the cut surface against the face of the block and then sectioned from the caudal thyroid cartilage through the epiglottis. Tissues were stained with haemotoxylin and eosin, Masson's trichrome, and Verhoeff-van Gieson according to standard procedures. Digitally scanned tissue elements were quantified for comparative analysis.

Results: Consistently reproducible, high quality microscopic slides of the rat and rabbit larynx were achieved with these tissue processing methods. Differences in collagen and elastin distribution were identified between the rat and rabbit vocal fold lamina propria.

Conclusion: Rats and rabbits are good animal models for laryngeal function; however, species differences should be considered in light of the investigative question and appropriate anatomical landmarks should be analyzed.

Impact statement: Quantifying the species-specific variations in anatomy and extracellular matrix protein content can help researchers better determine the ideal animal model for a particular laryngeal function study.
Juvenile Animal Studies in Support of Pediatric CNS Drugs: CDER’s Experience Ten Years Later

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Introduction: A recent survey was conducted to evaluate juvenile animal studies submitted to CDER between 2009 and 2014. Some conclusions about the nonclinical pediatric safety assessment based on studies conducted in support of CNS-active compounds are presented here. Methods: A total of 42 completed studies submitted to the Divisions of Psychiatry and Neurology Drug Products were evaluated. Data on species used, endpoints evaluated, and tests conducted to assess neurobehavioral outcomes were analyzed. Results: Of the drugs evaluated, all had studies conducted in rats, but in some cases a second study in a nonrodent species was also conducted (30%). The age at initiation of dosing for rat studies ranged from postnatal day 4-28 and the duration of dosing from 6-13 weeks. Indices of growth and development were always included and standard clinical pathology and histopathology were included in the majority (~80%) of studies. An expanded neurohistopathology evaluation was also generally conducted. Reproductive performance was evaluated in 86% of the studies. A variety of neurological and neurobehavioral tests were employed, but in the majority of studies the potential for long-term cognitive impairment was evaluated using a complex water maze. The results of these studies revealed effects that had not been seen previously in adults or for which juvenile animals were more sensitive. Case studies will be presented. Conclusion: Juvenile animal studies provided safety information considered relevant to drug use in children and that was included in labeling in 60% of the cases and had a regulatory impact in 88% of the cases.
Variability of Spleen and Mesenteric Lymph Node Pathology Parameters in Control Cynomolgus Monkeys (*Macaca fascicularis*): A Retrospective Assessment

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Histologic sections of spleen and mesenteric lymph node (LN) for 450 control cynomolgus monkeys from routine nonclinical safety studies were evaluated to determine the incidence of background findings. The resulting data set was analyzed for correlations with other study data, including terminal body weight, spleen weight, maturity, and peripheral lymphocyte count. Spleen weights were highly variable across the entire data set, including within a control group on a single study (up to 4.4-fold), and among animals with the same histologic finding. Similarly, microscopic observations were also variable within control groups. The most common light microscopic findings in the spleen were variation in the size and number of germinal centers, acidophilic material in lymphoid follicles, and compound lymphoid follicles. The most common light microscopic findings in the LN were eosinophilic infiltrates, variation in the size and number of germinal centers, and histiocytosis. The only meaningful correlation (correlation coefficient > 0.3), considering all parameters, was the positive correlation of the presence of malaria pigment with reticuloendothelial hyperplasia in the spleen (Spearman correlation coefficient r2=0.42). This data set demonstrates the inherent high variability of spleen and lymph node findings in routine monkey toxicology studies, and lack of correlation with other indicators of immune system change. In studies with typically low numbers of animals/group, spleen and lymph node parameters may not be reliable indicators of test article-related effects of immunomodulation or immunotoxicity, and should be interpreted with caution.
Hepatocellular Proliferation in the Sprague-Dawley Rat: Comparison of Three Proliferation Markers after a Two-Week Treatment Period Using a Potent PPAR Compound

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Introduction: many compounds induce liver enlargement in the rat, often as a result of hepatocellular hypertrophy and/or hyperplasia. In this study the mode of action of a chemical producing liver changes in rodents was investigated. Proliferation of hepatocytes and bile ducts was assessed in a preliminary mechanistic study, the performances of 3 proliferation markers being compared. Experimental Design: 5 male rats were exposed daily by gavage with the compound for 2 weeks. One control group received the vehicle under the same experimental conditions. BrdU was i.p. injected on Days 3, 11 and 14. Methods: FFPE liver sections were immunostained for BrdU, Ki67 and PCNA using Ventana automats, digitalized (x20) using an AT2 scanning device (Leica) and analyzed both quantitatively (using the ImageScope® software) and qualitatively. Quantitative results were normalized by tissue surface. Results: The compound induced marked liver weight increases and hepatocellular hypertrophy. Both quantitative and qualitative assessments led to similar conclusions: BrdU labeling proved to be sensitive to detect proliferation of hepatocytes after a 2-week treatment-period. Ki67 and PCNA immunostaining did not identify any increase in numbers of positive cells. Conclusion: Only BrDu labeling was sensitive enough to investigate proliferation of heptocytes in a 2-week study. Ki67 and PCNA were not adequate. Impact statement: when it comes to investigative studies with a special focus on cell proliferation, selection of the proliferation marker should thoroughly be discussed and validated in a preliminary study prior to starting the main assay. BrDu labelling may be more effective in estimating hepatocellular proliferation, despite being a more labor-intensive technique.
The Novel Microtubule Targeting Agent (MTA) DZ-2384 Is Less Neurotoxic to the Dorsal Root Ganglia (DRG) and Sciatic Nerves of Sprague-Dawley Rats in Comparison to Docetaxel

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Introduction: MTAs target the microtubules of the mitotic spindle, arrest cell division and trigger apoptosis in cancer cells. MTAs also disrupt microtubules of the neuronal cytoskeleton resulting in neuronal and axonal degeneration, which is often not completely reversible. Our objective was to compare the neuropathology of docetaxel to a novel MTA, DZ-2384 in the DRG and sciatic nerves. Experimental Design: Twelve female rats per group were treated once weekly for 4 weeks at 30 and 12 mg/m² for DZ-2384 and 120 and 60 mg/m² for docetaxel. Five animals per group underwent a 22-Day recovery period. Samples were fixed in 10% neutral buffered formalin, processed and stained with hematoxylin eosin-phloxine. Results: At day 24 following treatment, no microscopic evidence of neurotoxicity was present for DZ-2384 at 12 mg/m², while minimal degeneration was observed in the DRG neurons, DRG axons and sciatic nerve axons in 1/7, 2/7 and 3/7 rats respectively given 30 mg/m². In contrast, minimal degeneration was observed in the DRG neurons, DRG axons and sciatic nerve axons in 7/7, 4/7 and 7/7 rats, respectively at 60 mg/m² docetaxel, and minimal to moderate neurotoxicity was observed in all rats treated with 120 mg/m² docetaxel. Following the recovery period, no neurotoxicity was observed for DZ-2384 at either dose but minimal to mild neuronal degeneration remained for docetaxel and was dose proportional. Conclusion: At doses corresponding to the MTD, DZ-2384 resulted in less frequent, less severe and reversible neurotoxicity compared to docetaxel.
Immune Complex Glomerulopathy in Human CD19 Transgenic Mice

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Introduction: Unexpected morbidity at 41 weeks of age during a lifetime observational study with untreated HuCD19 transgenic (Tg) mice was determined to be due to glomerulopathy. Additional testing was added to the Protocol to characterize the renal changes.

Experimental design: 180 male HuCD19Tg mice were single housed, fed ad libitum, and allowed to live out their lifespan without additional treatments. Blood was collected at baseline, 26, 52, 78, and 108 weeks of age for analysis and subgroups of animal sacrificed and necropsied at the last three time points for histopathology. Urine was also collected at several time points after 50 weeks of age.

Methods: Blood samples were analyzed for hematology and several clinical chemistry parameters and urine for creatinine, micro albumin and micro total protein. H&E sections were prepared and examined for all tissues and transmission electron microscopy (TEM) and immunohistochemistry (IHC) were also performed on selected kidney blocks.

Results: Urinary parameters increased to 52 weeks of age and then declined to or below baseline with advancing age. Increased urinary microalbumin levels correlated with the severity of glomerulopathy at 76 and 84 weeks of age. Histopathology, TEM, and IHC demonstrated immune complexes containing C3, IgG, and IgM within glomeruli.

Conclusion: Immune complex glomerulopathy due to overexpression of CD19 led to early death of some HuCD19Tg mice.

Impact statement: HuCD19Tg mice remain an important model for any therapeutic directed against CD19, though longevity may be impacted by spontaneous immune complex glomerulopathy.
Corneal Lesions in Rats Induced by Intraperitoneal Injection of the Anesthetic Mixture of Three Drugs

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Introduction: The combination of medetomidine, midazolam and butorphanol by intraperitoneal injection has been introduced recently in experimental animal studies as an anesthetic agent. We encountered the corneal opacities in rats which had surgical operation of nephrectomy under this anesthesia. The purpose of this study was to examine whether similar changes were observed in normal rats.

Experimental Design and Method: SD rats had surgical operation of nephrectomy by this anesthetic agent at 4- and 5-week-age. Ophthalmic examinations were conducted for all animals, and they were necropsied 4 weeks after operation and evaluated histopathologically. Normal SD rats were set 2 groups (4- and 5-week-age, 5 animals/group) and were similarly administered this anesthetic agent on Days 1 and 16. Ophthalmic examinations were conducted for all animals, and they were necropsied on Day 30.

Result: In nephrectomized rats, corneal opacities were observed in 66/83 animals and the lesions were histopathologically consistent with the mineralization with focal inflammation in the corneal stroma. In normal rat groups, corneal opacities were first observed on Day 3 and these changes were observed in 4/5 to 5/5 animals on Day 29. In ophthalmic and gross examinations, the degree and size of corneal lesions in nephrectomized rats was more remarkable than those of normal rats.

Conclusion: Corneal opacities occurred in normal and nephrectomized rats when the anesthetic mixture of medetomidine, midazolam and butorphanol were treated.

Impact statement: The effect of corneal opacity should be taken into consideration when using the anesthetic mixture of medetomidine, midazolam and butorphanol in rats.
Pathophysiologica Analysis of Skeletal Muscle in Obese Type 2 Diabetes Spontaneously Diabetic Torii (SDT) Fatty Rats

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Sarcopenia is age-related decrease of muscle mass and function. Diabetes is one of the factors accelerating sarcopenia but details of the mechanism are still not clear. We report characteristics of the skeletal muscle of the Spontaneously Diabetic Torii (SDT) fatty rat, an obese type 2 diabetes rat model, and demonstrate the usefulness of this rat for analysis of the diabetes-related sarcopenia.

SDT fatty rats were sacrificed at 8, 16, 24, 32 and 40 weeks of age. Cross sections of the soleus (slow-twitch type I fiber) and the extensor digitorum longus (EDL) muscle (fast-twitch type II fiber) were examined histologically. Plasma IGF-1 levels and mRNA levels of Murf-1 and Atrogin-1 in the skeletal muscle were measured for analysis of the muscle protein synthesis and proteolysis, respectively. The muscle strength of the animals was also measured. Sprague-Dawley (SD) rats were used as normal control for each age.

Muscle weights of SDT fatty rats were much lower than those of SD rats for each age. Histologically, skeletal muscles of the SDT fatty rats showed the decreases in size of muscle fibers, especially in type II fibers. An increase in the intramuscular lipid was also observed in the SDT fatty rats compared with the SD rats. Plasma IGF-1 levels and muscle strength decreased in the SDT fatty rats at 24 weeks of age. These results suggested that sarcopenia-like changes occurred in the skeletal muscle of the SDT fatty rats in aging.

We conclude that SDT fatty rat is a useful model for analysis of diabetes-related sarcopenia.
Chondrodysplasia in a Stillborn Simmental Calf

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Introduction: Chondrodysplasia is a developmental bone disorder occurring due to primary defect in cartilage maturation. It is an inherited defect in many breeds of cattle resulting from a genetic mutation that affects the normal cartilage development. The mutation and the mode of inheritance are well characterized for Dexter and Angus breeds but are not known for other breeds including Simmental.

Experimental design: A pathological case report from a sporadic case.

Methods: Gross necropsy findings and histological changes in limb bones were evaluated.

Results: Gross necropsy finding included disproportionate dwarfism, a relatively large head, cleft palate, protruding tongue, and bilateral inguinal hernia. The axis of the brain was deviated approximately 90 degrees. There were severe shortening and curving of all long bones. The limb joints did not show significant laxity or ankylosis. Histologic examination of the growth plates of long bones revealed disorganized chondrocyte columns that lacked orderly alignment into distinct zones. There were irregular projections of partly calcified cartilages that protruded into the metaphysis without forming uniform regular spicules of primary spongiosa. Ossification was incomplete with large cores of partly calcified cartilage within the bony spicules. The medullary cavity contained markedly reduced hematopoietic cells.

Conclusion: The gross and histological features are consistent with congenital lethal chondrodysplasia (Dexter type ‘bulldog dwarf’ phenotype).

Impact statement: The pathology of chondrodysplasia is not well characterized for Simmental breed. This case report describes the pathology of chondrodysplasia and underscores the need for research to investigate an underlying cause.
Pharmacologic Inhibition of Nav 1.7 in Sprague-Dawley Rats Does Not Recapitulate the Anosmia-Associated Decrease in Tyrosine Hydroxylase Expression Observed in the Olfactory Bulb of cNav1.7−/− Mice

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Introduction: Loss of function of SCN9A, the gene encoding the voltage-gated sodium channel Nav1.7, causes a congenital inability to experience pain and Nav1.7 is essential for odor perception. In cNav1.7−/− mice, odor-evoked action potentials are generated in olfactory sensory neurons (OSNs), but they fail to transmit signals from their axon terminals within olfactory bulb glomeruli. In addition, tyrosine hydroxylase (TH) expression in juxtaglomerular neurons, a correlate of afferent trans-synaptic activity, is markedly reduced. Bouton and synapse morphology are unperturbed as demonstrated by electron microscopy and immunohistochemical staining of olfactory marker protein (OMP) and vesicular glutamate transporter 2 (vGluT2) in OSNs.

Experimental Design: To determine if this pattern was recapitulated via pharmacologic inhibition of Nav1.7, IHC was performed on olfactory bulb tissues from a standardly designed 26-week rat repeat dose toxicology study evaluating a potent Nav 1.7 inhibitor. Methods and Materials: FFPE olfactory bulb tissue was immunohistochemically stained with Anti-OMP, vGLUT2 and TH antibodies (Abcam, Inc.) on a Ventana Discovery XT Platform®. Intensity and frequency of staining were graded using 1-4 scales. Olfactory regions analyzed were inclusive of the internal plexiform/mitral, external plexiform and glomerular layers. Results: IHC staining was uniform and consistent between vehicle control and treated groups, indicating no treatment-related effects. Conclusion: TH expression was not diminished and thus synaptic transmission within the olfactory glomerulus appeared intact in rats treated with a potent Nav1.7 inhibitor. Impact Statement: Pharmacologic inhibition of Nav1.7 may not result in anosmia in a therapeutic setting.
Minipig Dermal Studies

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Miniature swine are increasingly used in biomedical research and are the preferred non-rodent species in preclinical dermal safety studies, as minipig skin has been reported to be similar to human skin. However, there is potentially a wide variance in skin morphology, depending on the region of the body sampled. Thus, selection and sampling of dosing and comparator control sites may introduce unwanted variance and skew histopathological assessment of test article-related findings. We developed a comprehensive map for skin site sampling, improved sampling methods to allow standardized assessment, and present here a rough comparison of sites. Seventeen separate sites were identified for sampling, including routinely utilized dosing and control sites. 3x3cm skin samples were taken post-euthanasia from each site for six adult Göttingen and six adult Yucatan minipigs. Various methods of fixation were assessed for consistency, including standard floatation, cloth bagging, and affixation to a waxed card with filter paper overlay. Samples were sectioned, stained, and evaluated for basic qualitative histoanatomical comparison. Fixation on a waxed card overlaid with filter paper was most effective in minimizing tissue distortion. There were notable differences in qualitative skin thickness between the commonly dosed dorsal sites and the ventral sites routinely used for skin sampling, with the dorsal sites having notably thicker dermis and subcutis. Careful site selection for dosing and sample handling of minipig skin should be considered to facilitate accurate assessment of compound-related effects in the research setting.
Integrating Immunopathology and Immunotoxicology to Evaluate Immunomodulatory Drugs

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Introduction/Objectives: The immune system is an important target when assessing toxicity but its complexity can make evaluating immunotoxicity difficult. Historically, simple assessment of immunosuppression, lymphocyte depletion and immune cell death were considered sufficient in safety assessment. However, with increasing immunomodulatory therapeutics being developed, it has become necessary to provide more complex evaluations of nuanced changes in immune response and integrate data from multiple disciplines (e.g. immunology, immunotoxicology, pathology). Pathologic evaluation is a necessary component to assess toxicity but in isolation, commonly provides limited information on immunotoxicity. Thus, it is necessary to evaluate and integrate immunotoxicology assessment into pathology assessment of the immune system. However, a lack of familiarity with assays and data interpretation of these assays often prevents integration between immunopathology and immunotoxicology data. Methods/Experimental Design/Results: Here, we provide experimental design and data sets from commonly employed immunotoxicology assay data (e.g. immunophenotyping, cytotoxicity assays, T-dependent antibody responses (TDAR), proliferation and cytokine release assays) to help provide a familiarity with assays that can be used to complement pathology findings and discuss integration of these data sets with pathology findings. Conclusion/Impact Statement: To fully assess the efficacy, preclinical safety and risk assessment of therapies impacting the immune system, a multi-tiered approach with significant communication between immunologists, pathologists and toxicologists throughout the drug development process is required. With the increasing array of immunomodulatory therapies, there is often a regulatory expectation that there will be integration across the disciplines of pathology, immunology and toxicology which necessitates early and continued communication during drug development.
Spontaneous Ectopic Choroid Plexus with Sclerosis in Adult Beagle Dogs in General Toxicity Studies

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Microscopic examination of the brain in four different general toxicity studies using adult beagle dogs revealed the presence of a spontaneous finding in the choroid plexus of 6 different individuals (4 females and 2 males, ages ranging from 12 to 18 months). This finding was characterized by a well circumscribed mass that was located above and along the corpus callosum and was composed of columnar ependymal cells forming tubular structures surrounded by variable amounts of fibrous connective tissue and blood vessels. The microscopic appearance of each mass was consistent with small rests of ependymal cells that had been penetrated by the leptomeninges during neural development but which had failed to migrate to the ventricles through choroid fissures (as would have occurred during normal development of the choroid plexus). The masses did not compress adjacent brain tissue and were not associated with any clinical signs. A morphologic diagnosis of ectopic choroid plexus with secondary sclerosis was made. To the authors' knowledge, ectopic choroid plexus has not been reported in beagle dogs and is rare in humans and horses.
Radiation Causes Delayed Injury in the Kidneys of Rhesus Macaques

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Introduction/Objectives: Fibrosis of various organs is a delayed effect of irradiation. Studies in rhesus macaques defined the morphologic progression of radiation-induced renal injury. This model will be used for testing the long-term benefit or consequences of therapy.

Experimental Design: Male rhesus macaques (N = 94) received acute, single-exposure irradiation at 10, 11 or 12 Gy. Shielding the lower limbs protected approximately 5% of the total body bone marrow. The animals were euthanized when dictated by clinical condition, or at approximately 180 days.

Methods and Materials: Kidney specimens were preserved in 10% formalin, processed by standard histologic techniques, and 5 µm sections stained with hematoxylin and eosin, Masson’s trichrome, toluidine blue, Perls’ iron stain, periodic acid-Schiff/hematoxylin, and immunohistochemical stains for α-smooth muscle actin (SMA) and tryptase.

Results: Radiation-associated glomerular changes included segmental congestion and/or thrombosis, aneurysmal capillary dilatation, increased mesangial matrix, fibrosis and atrophy. Congestion of corticomedullary vasculature was commonly noted. Tubules occasionally had hyaline droplet or vacuolar degeneration. Interstitial fibrosis often included myxoid cells containing metachromatic material on toluidine blue staining. SMA staining was commonly present in glomeruli and areas of interstitial fibrosis. Inflammatory cell infiltration was notably sparse.

Conclusions: Radiation-induced renal injury in rhesus macaques was associated with a spectrum of delayed, through 108d post-exposure, histologic changes in renal glomeruli, interstitium and tubules, as well as congestion of corticomedullary blood vessels.

Impact Statement: Delayed effects of kidney irradiation in rhesus macaques show that long-term health surveillance is indicated following accidental or belligerent radiation exposures.
Delayed Radiation-Associated Changes in the Lungs of Rhesus Macaques

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Introduction/Objectives: Fibrosis of various organs may be a delayed effect of irradiation. Studies of radiation injury in rhesus macaques defined the morphologic progression of the pulmonary injury. This model will be used as a platform for testing potential therapeutic interventions.

Experimental Design: Male rhesus macaques (N = 94) received acute, single-exposure irradiation at 10, 11 or 12 Gy. Shielding for the lower limbs protected approximately 5% of the total body bone marrow. The animals were euthanized when dictated by clinical condition, or at approximately 180 days.

Methods and Materials: Lung specimens were preserved in 10% formalin, processed by standard histologic techniques, and 5 µm sections stained with hematoxylin and eosin, Masson's trichrome, toluidine blue, and immunohistochemical stains for α-smooth muscle actin and tryptase.

Results: Radiation-associated pulmonary changes included interstitial and pleural fibrosis, alveolar edema, epithelialization of alveolar walls, alveolar macrophage aggregates, and infiltrations of mononuclear cells, eosinophils and neutrophils. Alveolar walls had myxoid cells containing metachromatic material on toluidine blue staining. Smooth muscle actin staining was present in areas of interstitial and pleural fibrosis. Prominent alveolo-bronchiolar hyperplasia with mucous cell metaplasia was noted in a few animals.

Conclusions: Irradiation of the lungs of rhesus macaques was associated with a spectrum of delayed histologic changes, including interstitial and pleural fibrosis, alveolar edema, alveolar macrophage aggregates, mixed inflammatory cell infiltration, myxoid cells in alveolar walls, and alveolo-bronchiolar hyperplasia.

Impact Statement: Late effects of lung irradiation in rhesus macaques suggest long-term health surveillance may be indicated following accidental or belligerent radiation exposures.
Acute and Prolonged Radiation-Associated Changes in the Gastrointestinal System of Rhesus Macaques

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Introduction/Objectives: Studies in rhesus macaques have defined the morphologic progression of radiation-induced intestinal injury, from acute damage through to long-term histopathological changes including fibrosis. This model will be used for testing the long-term benefit or consequences of therapy against radiation injury.

Experimental Design: Male rhesus macaques (N = 94) received acute, single-exposure irradiation at 10, 11 or 12 Gy. Shielding for the lower limbs protected approximately 5% of the total-body bone marrow. The animals were euthanized when dictated by clinical condition, or at approximately 180 days.

Methods: Intestinal specimens were preserved in 10% formalin, processed by standard histologic techniques, and 5 µm sections stained with hematoxylin and eosin, Masson’s trichrome, toluidine blue, periodic acid/alcian blue, and immunohistochemical stains for α-smooth muscle actin, tryptase, Ki-67, MxA, and lipopolysaccharide core antigen.

Results: Initial radiation-associated changes within the first month were dominated by mucosal depletion, followed by epithelial proliferation to restore the mucosal barrier. Delayed responses included fibroplasia/fibrosis of the submucosa, serosa and muscularis externa in a subset of animals. The mucosal architecture was not restored to normal levels through 180d post-exposure.

Conclusions: Radiation-induced intestinal injury included mucosal depletion and significant crypt damage followed by regenerative epithelial proliferation in the small and large intestine. Intestinal fibrosis occurred in a subset of animals.

Impact Statement: Delayed effects of intestinal irradiation in rhesus macaques show that long-term health surveillance is indicated following accidental or belligerent radiation exposures.
Aortic Tears in Nonclinical Research Rodents

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Aortic tears are very rare in control and treated rats and mice utilized for nonclinical safety assessment. This poster will present the microscopic features of two case examples in a control mouse and rat. The lesions are typically present within the ascending aorta just distal to the aortic valve and characterized by a sharply-demarcated separation of the intima and media lined by fibrin. Perivascular fibrosis, hemorrhage, and leukocytes typically surround the tears. None of the cases have evidence of esophageal or tracheal perforation. Similar findings in other studies, at this and other laboratories, have demonstrated a consistent location and histologic appearance of the aortic lesion with varying chronicity of perivascular findings. Aortic tears in rodents are considered extremely rare lesions, and since they can occur in control animals and/or multiple animals without a dose relationship, they are considered to be a complication of handling and/or the gavage procedure. This is the first known report of this finding in untreated control rodents. Monitoring and investigative studies are being conducted to further elucidate the pathogenesis in order to implement preventative measures. Toxicologic pathologists should consider handling/gavage procedure as a potential cause of aortic tears in rodents on safety evaluation studies.
Diversity of Thymic Cysts in Cynomolgus Monkeys

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Thymic cysts have been reported in several animal species, including non-human primates. In most species, they are located in the cortex or medulla and either congenital or acquired. Increased cyst formation accompanies physiological involution in some species. A survey was done on 130 Asian purpose bred cynomolgus monkeys, from 16 safety assessment studies conducted between 2015 and 2016. H&E stained thymus and gonadal tissues from males and females from control groups were re-examined microscopically. An eyepiece micrometer was used for measuring cysts. The incidence of the thymic cysts was evaluated with respect to maturity (gonadal), thymic involution, size, distribution, and morphology. Thymuses from Asian monkeys were from sexually immature animals (40%), maturing (9.2%) or mature (50.8%) animals. Examined thymuses showed no involution (53%), minimal (16.2%), slight (20.7%), moderate (6.2%) or marked (3.9%) involution. A total of 63 Asian monkeys (48.5%) had thymic cysts. The incidence of cysts (but not the number) increased with the severity of the involution. Larger cysts were present in thymuses with increased number of cysts but did not correlate with the severity of involution. Cysts were present predominantly in the medulla, septum, and capsule (alone or a combination of two), and less often in the cortex or corticomedullary junction. Cysts were lined by one to two layers of flat, cuboidal or squamous epithelium, and contained luminal floccular material, foamy macrophages, and occasional inflammatory cells. The distribution and morphology suggests that thymic cysts in cynomolgus monkeys may have different origins.
Animal Model of Bronchopulmonary Dysplasia in Juvenile Rats

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Introduction: The animal model for bronchopulmonary dysplasia, a debilitating condition in preterm human infants, is produced by the administration of 95% oxygen to neonatal and juvenile rats. Optimal fixation of these lungs is imperative to determine efficacy of potential therapy for this condition. Experimental Design: Neonatal rat pups were exposed to 95% hyperoxic conditions by whole-body inhalation continuously for 14 days. Materials and Methods: At necropsy on postnatal day (PND) 15, lungs were fixed in formalin using either immersion, intratracheal vapor or intratracheal liquid over various timeframes and pressures. Optimal fixation was determined by evaluating H&E stained sections for the inflammatory component (alveolitis) and the dysplastic component (alveolar simplification) of bronchopulmonary dysplasia. Mean chord length (Lm) and quantity of ED-1+ macrophages were determined by 2-dimensional morphometry using Visopharm software and immunohistochemically stained tissues. Heart weight was recorded, and mean right ventricular weight (Fulton’s Index) was calculated. Results and Conclusion: Intratracheal infusion of liquid formalin for 30 minutes at 25 cm H₂O was optimal to uniformly inflate bronchodysplastic lungs of the juvenile rat in order to: i) best identify the histopathologic components of bronchopulmonary dysplasia; ii) accurately assess the severity of inflammation; and iii) detect intergroup differences in the numbers of ED-1 macrophages and Lm. Increased right ventricular weight as determined by Fulton’s index confirmed the validity of this model system. Impact statement: This hyperoxic rat model along with optimal fixation of young dysplastic lungs can be appropriately used to assess the efficacy of therapies to treat and/or prevent bronchopulmonary dysplasia in preterm newborns.
Thyroid Hormone Measurement in Rats, Correlation of Histopathology, and Possible Decision Tree for OECD 421/422/443

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Introduction: As new OECD test guidelines 421/422/443 are released, thyroid hormone measurements in rats with commercially available test kits and correlation to histopathology are now mandatory.

Experimental Design: Blood samples from PND4, PND13 and/or PND22 and adult animals in different study types (OECD 421/422/443) were taken. Additionally, thyroid glands were preserved in 10% neutral buffered formalin.

Methods: ELISA kits for measurement of T3 and T4 in rats were validated and compared among each other regarding precision and functional sensitivity with established radioimmunoassays (RIA). Measurements of T3 and/or T4 and TSH in PND4, PND 13, PND 22 pups or adult rats. and concurrent thyroid gland histopathology were performed. Thyroid gland weight was carried out in adult rats.

Results: Inter-individual variation of hormone values in control pups were assessed and statistical power was calculated. These parameters were compared with corresponding values observed in adult rats. In pups until PND 22 no sex-specific differences of thyroid hormone levels occurred. In case of relevant thyroid hormone changes concurrent histopathological findings of thyroid gland were seen. No histopathological correlate was observed with T4 decreases in PND13 pups.

Conclusion: Assessment of thyroid hormone measurements should be done in correlation with pathology (organ weights, histopathology) and in a step-wise approach following a decision tree.

Impact statement: The measurement of thyroid hormones in combination with pathology in the various cohorts in OECD TG 421/422/443 guideline studies should follow a defined step-wise approach.
Drug-Induced Fluorosis in Skeletal/Dental Tissues in Rodent Chronic Toxicity Studies

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Introduction: In a chronic toxicity study in rats, characteristic findings in bone and teeth were identified, including bone fracture, incisor discoloration, histopathologic pigmentation, decreased trabecular bone, and ameloblast degeneration. Based on similarities of these changes to those seen in fluorosis in rats, investigational studies were conducted. Experimental design: Compound A was administered orally by gavage for up to 14 weeks in rats. Pathologic examination and fluoride measurements in urine and bone were conducted. A chronic study was also conducted in a similar manner with sodium fluoride (NaF), a compound known to induce fluorosis, as a positive control. Results: Bone changes were characterized by decreased trabecular bone, increased osteoid, and artifactual fluoride-related pigmentation, correlated with decreases in strength and mineral density. In the incisors, degeneration of the ameloblast with decreased iron content was associated with discoloration. These changes were fully consistent with those noted in rats given NaF that have developed fluorosis. Significant increases in fluoride in urine and bone were observed in rats treated with compound A, and a high ratio of defluorinated-metabolites was identified. The release of fluoride from compound A was further corroborated by inhibition of urinary fluoride excretion following co-administration of a non-selective CYP inhibitor. The bone/teeth toxicity induced by compound A is rodent specific. Conclusion: This is the first report, to our knowledge, to demonstrate in rats that the skeletal and dental toxicity noted in long term toxicity studies was due to fluoride released through metabolism of the compound.
Molecular Pathology Approach to Characterize Unique Distribution of Drug-Induced Phospholipidosis in Rats

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In order to support preclinical development of an investigational anti-cancer compound, Sprague-Dawley rats (10/group + 5/recovery group) received oral gavage doses of Compound at 0, 25, 50, or 100 mg/kg/day for 28 days followed by a 28-day recovery period for the control and high-dose groups. At ≥50 mg/kg/day, histopathology findings of vacuolated histiocytes or vacuolated parenchymal cells was observed in the lungs, kidneys, liver, and blood vessels of pancreas, skin and lungs. The microscopic findings were consistent with multi-organ phospholipidosis (PLD). PLD in blood vessels was generally associated with secondary inflammatory changes such as edema and inflammatory infiltrates around blood vessels. Immunohistochemical staining and electron microscopic examination was performed. Using molecular pathology tools, the intracellular presence of phospholipid whorls and their co-occurrence with endothelial cell-specific markers within the vascular wall confirmed the diagnosis of vascular PLD. Marked intimal and adventitial thickening of the vessels was due to an unusual presentation of PLD. Following 1-month recovery period, vascular findings were seen in fewer tissues with lower incidence and severity suggesting a partial recovery. These vascular findings were considered part of multi-organ PLD and not a direct vascular effect of the compound. This study provides a previously unreported presentation of drug induced PLD in the rats. Vascular PLD should be considered as differential diagnoses for vacuolated vascular/perivascular cells.
Immunohistochemical Characterization of Ocular Inflammation Associated with Intravitreal Biotherapeutics

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Introduction: Ocular inflammation in preclinical intravitreal (IVT) studies with biotherapeutics represents a potential safety hazard, but the mechanism is poorly understood. Within the affected eyes, the inflammatory cells are typically perivascular with/without vasculitis. The goals of this study were to immunophenotype ocular inflammatory cell infiltrates and compare its relationship to anti-drug antibody (ADA) response. Experimental Design: Formalin-fixed paraffin embedded (FFPE) cynomolgus eyes from six non-GLP dose range finding IVT studies for antibody-based biotherapeutics were used. Methods: Immunohistochemistry for immune cells (CD3, CD4, CD8, CD68, CD19), pericytes, SMA and immune complex deposits (IgG, IgM, complement C3b) was performed. Results: T cells, B cells, and macrophages with variable severity score (0-3+) were localized in all cynomolgus eyes with biotherapeutics-related inflammation. Spatially, the inflammatory cells were most common in the ciliary body, followed by iris, choroid, retina and optic nerve head, and occasionally in the sclera. Visible granular immune complexes, as evidenced by IgG, IgM or C3b immunostains, were not detected, although there were positive correlations between ocular inflammation and ADA in animals from each of the 3 studies where serum ADA was measured. Conclusions: IVT biotherapeutics-related ocular inflammation in cynomolgus monkeys is not characterized by large immune complex deposits, in spite of serum ADA. This could indicate different mechanisms compared to systemic immune-mediated vascular inflammation. Impact statement: Further characterization of inflammatory cell infiltrates in the context of ocular immune privilege and no obvious large immune complex deposits may help us better understand the pathogenesis of IVT biotherapeutics-related ocular inflammation.
Histological Features of the Nasal Cavity in the Juvenile White Rabbit

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Introduction: Rabbits are one of the experimental animals used in ocular or inhalation toxicology studies. Nasal cavity is a relatively highly exposure site of ophthalmic or inhalational drugs via the nasolacrimal duct or by inhalation; however, little information is currently available. To provide the information, histological characteristics of the nasal cavity in the neonatal/infantile rabbits were investigated.

Experimental Design: Eleven neonates/infants of Japanese white rabbits Kbl:JW were euthanized each in turn at postnatal day 1-24 every 2-4 days. Following euthanasia, their skulls were fixed and decalcified. H&E stained transverse sections were prepared and examined microscopically. Results: During the postnatal period, nasal airway components and around tissues were developing, together with enlargement of the nasal cavity. Turbinate was elongated and branched with chondral proliferation and was formed with endochondral ossification, while bones composed of the nasal cavity wall were formed by intramembranous ossification. Squamous, transitional, respiratory, and olfactory epithelia in the neonates were distributed on the surface of the nasal cavity, similarly to those in the adults. Below the nasal cavity, deciduous incisors and molar/premolar teeth appeared in the neonatal period, which were replaced by permanent teeth with aging. Conclusion: the rabbit nasal cavity was developing during the postnatal period, in which the maxillary bone/cartilage formation contributed to its development. Impact Statement: This result provides basic knowledge for histological examination of the nasal cavity of juvenile rabbits and rationale for appropriate study design in juvenile toxicology studies, especially for ophthalmic or inhalational drug evaluation.
Effects of Scopolamine or SCH 23390 on Two-Way Active Avoidance Test and Expressions of Neuronal Cell Activity Marker at the Hippocampus and Amygdala in Rats

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Introduction: Learning test (two-way active avoidance test) was conducted in male rats administered with nonselective muscarinic antagonist scopolamine or selective dopamine D1-like receptor antagonist SCH 23390. Gene expressions of neuronal cell activity marker at the hippocampus and amygdala were evaluated using histopathological sections and brain slices. Experimental Design: Scopolamine or SCH 23390 was administered intraperitoneally at dose levels of 2.0 or 0.1 mg/kg, respectively for 4 days. Saline was administered in control rats. Learning test was performed during the dosing period. Brain slices were collected on the second day of dosing and neuronal cell activity marker; Arc mRNA expressions were measured using FISH and RT-qPCR. Results: In the learning test, the scopolamine-treated group showed comparable levels of avoidance rate to those of the control, whereas decreased freezing was observed. The SCH 23390-treated group showed low levels of avoidance rate and increased freezing. In FISH and RT-qPCR assays, neuronal Arc mRNA decreased at the hippocampus and amygdala in the scopolamine-treated group, and increased at the hippocampus in the SCH 23390-treated group. Conclusion: The result of learning test indicated inhibition of fear or context conditioning in the scopolamine- or SCH 23390-treated groups. Inhibition of context conditioning was not suggested by scopolamine treatment from our results. The expression of neuronal cell activity markers in the hippocampus and amygdala did not correlate with the results of learning test. Impact statement: Based on our experiment, the results of neuronal cell activity markers should be carefully interpreted in behavioral toxicity studies, especially in learning tests.
Diuretic-Induced Bladder Wall Hypertrophy in Rats—Evaluation by Image Analysis

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Objective: Diuresis can cause bladder hypertrophy in diabetic- and osmotic diuretic-induced rats. We investigated whether increased urine volume would induce bladder wall hypertrophy by image analysis of the urinary bladder. Experimental design: Male Crl:CD(SD) rats were dosed twice daily with oral doses of 0, 30 and 100 mg/kg of furosemide (Group: control, 60 mg/kg/day, 200 mg/kg/day), and sacrificed at 1 week (n=6 per dose) or 4 weeks (n=6) post dosing, or at 4 weeks (n=3) after the recovery period. Methods: Digital images of whole cross-sections of the urinary bladder were saved for analysis, and the images were analyzed with WinROOF (Mitani Corporation). The HE-stained slides were used to determine the bladder tissue cross-sectional area, and the cytokeratin-stained slides for the urothelium area, the smooth muscle actin-stained slides for smooth muscle area and sirius red-stained slides for the collagen area, respectively. Results: In the image analysis, no changes were observed at 1 week after dosing. At 4 weeks no significant changes were observed in the 60 mg/kg/day group. In the 200 mg/kg/day group, significant increases in the total bladder wall area and smooth muscle area were observed, which suggested that the increased bladder wall area were due to increased smooth muscle. Conclusion: Image analyses provided a reproducible and convenient method for detailed morphometric analysis of the bladder tissues. Impact statement: Using this method we observed increased total bladder wall area due to increased smooth muscle in the rats after the 4-week oral dosing of furosemide, indicating that furosemide induced bladder wall hypertrophy.