Juvenile Toxicity Testing in Non-Human Primates

Alys Bradley
Gary J. Chellman
Animal Models in Juvenile Toxicity Testing

Three criteria for species selection:

1. Species cross-reactivity
   - Acceptable level of reactivity
   - Similar tissue distribution

2. Pharmacology/efficacy

3. Immunogenicity
   - Anti-Drug Antibody (ADA)
     - Formation
     - Clearing
   - Neutralising of cross-reactive neutralising antibodies
Juvenile Toxicity Testing in NHPs

- Regulatory authorities are requiring Sponsors to consider paediatric indications for therapeutics in development.
- Juvenile toxicity studies should only be considered if existing animal and human data is insufficient to support paediatric studies, e.g. can’t use adult NHP data as difference in physiology and metabolism can alter sensitivity to drug effects.
- Paediatric investigation now mandatory for New Drug Applications in North America (FDA, 2006) and or Marketing Authorisation in Europe (EMEA, 2008).
Juvenile Toxicity Testing in NHPs

- Juvenile studies should only be used to test whether there are major developmental events that could occur in the paediatric patients that have not been examined in other preclinical NHP toxicity studies.

- Each study needs to be designed based on the specific concern being addressed.

- For biologics the NHP may be the only pharmacologically relevant species for toxicity testing.
## Organ Systems of Concern

<table>
<thead>
<tr>
<th>System</th>
<th>Human Data</th>
<th>Macaque</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immune system</td>
<td>development up to 12 yo</td>
<td>Development up to 6 mo</td>
</tr>
<tr>
<td>Reproduction</td>
<td>development up to adulthood</td>
<td>development up to adulthood</td>
</tr>
<tr>
<td>Skeleton (growth)</td>
<td>development up to adulthood</td>
<td>Well developed at birth (equivalent to 6yo human); mature at 6yo</td>
</tr>
<tr>
<td>Renal</td>
<td>development up to 1 yo</td>
<td>Complete at birth; grows in size not maturity</td>
</tr>
<tr>
<td>Pulmonary</td>
<td>development up to 2 yo</td>
<td>Complete at birth; grows in size not maturity</td>
</tr>
<tr>
<td>Cardiovascular</td>
<td>development up to 5-7 yo</td>
<td>Complete at birth; grows in size not maturity</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>development up to 1-2 yo</td>
<td>development up to 1-2 yo</td>
</tr>
</tbody>
</table>
Juvenile Toxicity Testing in NHPs

- At the time of birth, most organ systems in macaques are either as developed or more developed than in humans.

- Some of the events occurring postnatally in humans have already occurred prenatally in macaques, and have been assessed already in a developmental study ………no need for an additional juvenile study.

- Therefore only needed if the therapeutic agent is intended to be administered to paediatric patients.
Juvenile Studies in NHPs

- No such thing as a standard study

- Dose selection for NHP usually based on GenTox data

- Endpoints/design based on adult toxicity findings and/or any specific organ system concern

- Dose duration adequate to support clinical plan

- For monoclonals to treat chronic disease indications, the preferred design is an enhanced pre- and post-natal study in which pregnant monkeys are dosed from the beginning of organogenesis through parturition

Weinbauer GF, Chellman GJ, Rasmussen AD, Vogelwedde E
Chapter 13: Use of Primate Paediatric Model
Most Common NHP Models

- **Cynomolgus** (*Macaca fascicularis*)
  - Commonly used model
  - Year long fertility

- **Rhesus** (*Macaca mulatta*)
  - Much less frequently used than cynomolgus
  - Seasonal breeder

- **Marmoset** (*Callithrix jaccus*)
  - Lack of historical data
  - Hormone patterns different from human
  - Typically not used in general toxicology program
Challenges of Using NHP for Juvenile Toxicity Studies

- Small sample size (n = 10-20/group)
- Cost of studies
- Limited availability of CROs with expertise
- Low conception rate (30-40%)
- Long duration (gestation = 5.5 months)
- High abortion rate (15-20% full term)
- Single offspring
## Comparison of Rodent and NHP Studies

<table>
<thead>
<tr>
<th>Factor</th>
<th>Rat</th>
<th>Non-Human Primate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal availability</td>
<td>Routine</td>
<td>Can be a problem</td>
</tr>
<tr>
<td>Pre-weaning procedures</td>
<td>Routine</td>
<td>Can be (but not typically) done</td>
</tr>
<tr>
<td>Age of sexual maturity</td>
<td>9 to 13 weeks</td>
<td>4 to 6 years</td>
</tr>
<tr>
<td>Study/Group sizes</td>
<td>10 to 20 litters</td>
<td>5 to 10 juveniles</td>
</tr>
</tbody>
</table>
## Age Definitions - what is a juvenile?

<table>
<thead>
<tr>
<th>Age Groups</th>
<th>Human</th>
<th>Rat (mouse) (days)</th>
<th>Rabbit (weeks)</th>
<th>NHP (Cyno) (months)</th>
<th>Mini-pig (days)</th>
<th>Dog (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Premature</strong></td>
<td>less than term</td>
<td>1 to 4</td>
<td>0 to 1-2</td>
<td>-</td>
<td>-</td>
<td>1 to 4/10</td>
</tr>
<tr>
<td><strong>Neonate</strong></td>
<td>birth to 1 month</td>
<td>4 to 7/14</td>
<td>2 to 3</td>
<td>birth to 0.5</td>
<td>0 to 14</td>
<td>5/11 to 21</td>
</tr>
<tr>
<td><strong>Infant</strong></td>
<td>1 month to 2 years</td>
<td>7/14 to 21</td>
<td>3 to 5</td>
<td>0.5 to 5</td>
<td>15 to 28</td>
<td>22 to 42</td>
</tr>
<tr>
<td><strong>Children</strong></td>
<td>2 to 12 years</td>
<td>21 to 28F/35M</td>
<td>5 to 13</td>
<td>6-35</td>
<td>29 to 108</td>
<td>43 to 140F/170M</td>
</tr>
<tr>
<td><strong>Juvenile</strong></td>
<td>12 to 16 years</td>
<td>28F/35M to 49F/70M</td>
<td>13 to 21</td>
<td>36 to 48</td>
<td>120 to 180</td>
<td>150F/180M to 250F/260M</td>
</tr>
</tbody>
</table>
Availability of young NHP

- Often requested (by Sponsors and/or Regulatory Agencies) to use monkeys that are approximately six months old – *infant/young child*
- Suppliers will typically not ship before nine months old
  - Weaning must have occurred
  - Shipping younger may be possible, but with mothers
  - International shipment age can be six months older (IATA)
  - NHPs usually at least 10 to 12 months old at study start (quarantine, etc.) *child*
- Challenging to get the number, gender mix and appropriate age
Ages and Availability of Juvenile NHP

- **12 months** – Yearlings weigh ~1.3 kg *child*
- **12-14 months** – *child* weaning complete in the wild
- **CAVE:** Artificial weaning (permanent separation of infants from their mothers) should not occur before 10-14 months of age
- Infants separated early from the mother show development abnormalities, including:
  - impaired cognition
  - altered physiology
  - disrupted endocrine response to stress
  - behavioural abnormalities
  - inability to function in a social group
Ages and Availability of Juvenile NHP

- <10-12 months old available only two ways:
  - Purpose-breeding (Cost?!)
  - Spin-off from PPN Study

- If conduct Juvenile Tox as spinoff from Pre and Post Natal Development Study:
  - Controls: small ‘n’; uncertain gender mix
  - Treated: exposed to test article in utero
Ages and Availability of Juvenile NHP

18 months old child
• In the wild, juveniles usually remain close to their mother until the birth of a sibling

2 years old child
• Juveniles develop social skills through play.
• Diet now resembles that of adults
• Cynos and Rhesus are omnivorous frugivores, with dietary composition varying greatly with location and season
Ages and Availability of Juvenile NHP

• What Age of NHP do you really need? Testing juveniles or infants?
• Is the goal to close the gap below standard age for Repeat Dose Tox Study?
  – PPN (6m – 1 yo) to RD Tox Study (2-5 yo)
    [Gap depends on duration of infant exposure in PPN]
  – Current “fashion” to use sexually mature NHPs for standard tox widens gap (4-6 yo)
• Use of sexually immature NHPs to start chronic toxicity studies may eliminate the need for an additional study in juvenile NHPs
# Routes of Administration

<table>
<thead>
<tr>
<th>DOSE ROUTE (Earliest Day Post-Partum)</th>
<th>SPECIES</th>
<th>Rat</th>
<th>Mouse</th>
<th>Rabbit</th>
<th>Dog</th>
<th>Mini-pig</th>
<th>NHP†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral gavage</td>
<td></td>
<td>1</td>
<td>7</td>
<td>7</td>
<td>1</td>
<td>1</td>
<td>&gt;2 weeks</td>
</tr>
<tr>
<td>Intravenous bolus</td>
<td></td>
<td>4 to 15</td>
<td>10</td>
<td>14</td>
<td>4 to 14</td>
<td>-</td>
<td>&gt;2 weeks</td>
</tr>
<tr>
<td>Intravenous infusion‡</td>
<td></td>
<td>28</td>
<td>?</td>
<td>?</td>
<td>49 to 56</td>
<td>28(7)?</td>
<td>&gt;2 weeks</td>
</tr>
<tr>
<td>Inhalation</td>
<td></td>
<td>4 to 7</td>
<td>21</td>
<td>?(10)</td>
<td>10</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>Parenteral (IM/SC)</td>
<td></td>
<td>1</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>&gt;2 weeks</td>
</tr>
<tr>
<td>Dermal</td>
<td></td>
<td>10</td>
<td>21</td>
<td>35</td>
<td>42</td>
<td>28</td>
<td>&gt;2 weeks</td>
</tr>
</tbody>
</table>

Gradually improving ages, frequency and volumes

? = Unknown

† NHP’s younger than 9 months old need to be co-housed with mothers
‡ Chaired 30 mins to 1 hour
## Inhaled Therapies – Points to Consider

### Comparative Ages of Lung Development
### Interspecies Comparison of Alveolar Development

<table>
<thead>
<tr>
<th>Species</th>
<th>Onset</th>
<th>At Birth</th>
<th>Critical Period</th>
<th>Completion</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Human</strong></td>
<td>GWK36</td>
<td>Beginning</td>
<td>Birth – 2 yrs</td>
<td>6 – 8 yrs</td>
</tr>
<tr>
<td><strong>Dog</strong></td>
<td>?</td>
<td>Beginning</td>
<td>0 – 16 weeks</td>
<td>16 weeks</td>
</tr>
<tr>
<td><strong>Monkey</strong></td>
<td>?</td>
<td>Completed</td>
<td>in utero</td>
<td>Birth</td>
</tr>
<tr>
<td><strong>Rat</strong></td>
<td>PND 4</td>
<td>Not Started</td>
<td>4 – 14 days</td>
<td>3 weeks</td>
</tr>
<tr>
<td><strong>Rabbit</strong></td>
<td>?</td>
<td>Beginning</td>
<td>?</td>
<td>6 weeks</td>
</tr>
<tr>
<td><strong>Ferret</strong></td>
<td>?</td>
<td>Not Started</td>
<td>?</td>
<td>6 months</td>
</tr>
<tr>
<td><strong>Mouse</strong></td>
<td>PND 4</td>
<td>Not Started</td>
<td>3 – 14 days</td>
<td>3 weeks</td>
</tr>
<tr>
<td><strong>Minipig</strong></td>
<td>?</td>
<td>Completed</td>
<td>?</td>
<td>Birth (?)</td>
</tr>
</tbody>
</table>

*Modified from Zoetis, 1999*
EXAMPLE STUDY DESIGN
Juvenile NHP Toxicology Study Design
(13-week duration common)

- Clinical signs
- Body weight
- Ophthalmology
- Clinical pathology
- TK/ADA
- Skeletal
- Immunology
- Cardiology
- Behavioral

Week 13
Recovery

- Necropsy
- Organ weights
- Gross and histopathology
- Immunohistochemistry
- Bone densitometry

Typical age of juvenile NHP is 12-24M
Typical “n” 5/sex/group (3Term/2 Rec)
Evaluation of Study Endpoints

- Routine evaluations as per Repeat Dose Toxicity Study

- Detect any increased vulnerability of the immature monkey to the systemic toxicity of the drug compared with the adult

- Assess adverse influences on growth and development

- FT or Evaluations of the development of organ systems of concern
  - Those that undergo prolonged postnatal development or
  - Known treatment related effects in adult monkeys

- Reversibility (post-dosing development)
## Routine Evaluations

**FDA/EMA**

### EARLIEST DAY (Post-Partum)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>SPECIES</th>
<th>Rat</th>
<th>Mouse</th>
<th>Rabbit</th>
<th>Dog</th>
<th>Cyno</th>
<th>Minipig</th>
</tr>
</thead>
<tbody>
<tr>
<td>In-Life (clinical signs, body weight)</td>
<td></td>
<td>1</td>
<td>1</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Food consumption</td>
<td></td>
<td>22</td>
<td>22</td>
<td>28</td>
<td>42</td>
<td>180</td>
<td>28</td>
</tr>
<tr>
<td>Clinical pathology</td>
<td></td>
<td>1</td>
<td>1</td>
<td>5</td>
<td>1</td>
<td>14</td>
<td>1</td>
</tr>
<tr>
<td>Ophthalmology</td>
<td></td>
<td>21</td>
<td>21</td>
<td>21</td>
<td>21</td>
<td>14</td>
<td>7</td>
</tr>
<tr>
<td>Toxicokinetic sampling</td>
<td></td>
<td>1</td>
<td>1</td>
<td>5</td>
<td>1</td>
<td>14</td>
<td>1</td>
</tr>
<tr>
<td>Organ wt, gross &amp; microscopic obs</td>
<td></td>
<td>1</td>
<td>1</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>
Neonatal Evaluations

— Body weight
— Physical exam
  • inspection of the hard palate
  • spinal cord closure
  • digits, limbs, and joint angles for normal structural morphology
  • lymph node palpations
  • heart/respiration rate
Infant Measurements

- Clin Obs (daily)
- Body weight (weekly)
- Neurobehavior – BD7 and BD14
- External morphology
- Skeletal X-ray – BD28
- TK/ADA
- Flow Cytometry/TDAR
Juvenile Measurements

(Child/adolescent)

- Clin Obs (daily)
- Body weight (weekly)
- Neurobehavior
  - Modified human pediatric screens
  - Observational battery and neurological exam
- External morphology
- Skeletal X-ray
- TK/ADA
- Flow Cytometry/TDAR
Neurobehavioural Assessment

- Modified Irwin test
- Perform in a quiet room with minimum personnel
- Perform prior to any procedures that can cause stress/discomfort such as blood sampling, ophthalmology
- Higher-level cognitive and behavioural processes
- Higher inter-individual behavioural variability
  - Normal behaviour has to be established by a detailed knowledge of each individual animal
  - Need to integrate neurobehavioural responses with clinical signs
  - Take into account dynamic interactions with other social group members
  - Presence of normal vocalisation
Neurobehavioural Assessment

– Animal inside home cage (observations only) 
  • Posture; general locomotor activity; balance and coordination; alertness/arousal;
  • Vocalisations; grooming; tremors; convulsions; twitches/jerking; unusual behaviour/stereotypy; salivation; lachrymation; ptosis; piloerection; mucosae abnormal; urination & defaecation (absent/present); retching; vomiting; startle response; aggressiveness

– Animal outside home cage (hand restraint):
  • Ocular motility; ocular position/symmetry; pupil diameter; pupillary reflex; blink reflex; limb muscle tone; extensor reflex; flexor reflex; respiration rate; depth of respiration; fine motor control and grip

(Moscardo, 2010)
## Safety Pharmacology measurements in Juvenile animals

<table>
<thead>
<tr>
<th></th>
<th>Mini-pig</th>
<th>NHP</th>
<th>Dog</th>
<th>Rat</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cardiovascular</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ECG</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>(heart rate)</td>
</tr>
<tr>
<td>Blood pressure</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Indirect</td>
<td>?</td>
<td>Yes</td>
<td>Yes</td>
<td>?</td>
</tr>
<tr>
<td>- Direct</td>
<td>?</td>
<td>?</td>
<td>Yes*</td>
<td>Yes*</td>
</tr>
<tr>
<td><strong>Pulmonary</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Respiratory minute vol.</td>
<td>?</td>
<td>?</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Resistance/compliance</strong></td>
<td>No</td>
<td>No</td>
<td>Technically challenging</td>
<td>No</td>
</tr>
</tbody>
</table>

* Post-weaning
# Physiology/Pharmacokinetics in Neonate/infant compared to Adult

<table>
<thead>
<tr>
<th></th>
<th>Neonate/Infant</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>GI Motility/pH</strong></td>
<td>Lower motility, Increased GI Absorption, Higher pH so increased absorption of basic molecule and decreased absorption of acidic molecules</td>
</tr>
<tr>
<td><strong>Protein Binding</strong></td>
<td>Lower, more free compound</td>
</tr>
<tr>
<td><strong>Total Water Content</strong></td>
<td>Higher, affects volume of distribution</td>
</tr>
<tr>
<td><strong>Metabolism</strong></td>
<td>Enzymes not fully developed so affects pharmacokinetics</td>
</tr>
<tr>
<td><strong>Glomerular Filtration</strong></td>
<td>Lower</td>
</tr>
<tr>
<td><strong>Biliary excretion</strong></td>
<td>Lower</td>
</tr>
</tbody>
</table>
Importance of Metabolism/Toxicokinetics

• Neonate to juvenile to adult, often dramatic differences
  – 0.2 x adult rates in human neonates
  – 6 x adult rates in children

• Nonclinical changes with age
  – differences in AUC up to 300-fold between days 7 to 28 post-partum

• Sample blood/plasma/serum from all ages
  – Micro-sampling from various sites at different ages

• Necropsy to sample tissue from all ages
Terminal Procedures

- Routine Histopathology
- Electron microscopy (e.g., liver)
- Morphometry (e.g., lung)
- Bone pathology, mechanics
- Neuropathology (CNS and PNS)
  - Whole-body perfusion with gluteraldehyde
  - CNS
    - Paraffin embedding, special stains
  - CNS/PNS
    - Plastic embedding, semi-thin sectioning, toluidine blue staining
Specialized Data Sets available in NHP

- Immunology
  - Immunoglobulins
  - Flow Cytometry
  - TDAR

- Bone
  - Physical or radiographic length
  - Density – DXA
  - Architecture – pQCT
  - Strength
Immunology

Toxicology Screen (Tier I)
- Haematology
- Immuno-histopathology

Immunology Screen (Tier II)
- Phenotyping
- NK cell activity
- T-cell dependent anti-body response (TDAR) to keyhole limpet hemocyanin (KLH); Cytokines, DTH

Species
- Rats, mice
- NHP (mini-pigs and dogs)
Assessment of Immune System

- Clinical Pathology
- Lymphocyte subsets
- Organ weights and Histopathology (Spleen, Thymus, Lymph nodes, Liver, Kidney)
- Functional tests of cellular and humoral immunity e.g. TDAR
Infant Immunology Measurements

• TK/ADA/Flow
  – Typically 3-4 timepoints
  – Flow Cell Populations
    • B cells, T-total, T-helper, T-cytotoxic, NK cells, & Monocytes
    • Juvenile Cynos similar subset proportions to adults

• TDAR
  – KLH Challenge at BD138 (primary) and BD152 (secondary): IgG and IgM at 7, 14 and 21 days

• Immunoglobulins
  – Total IgG decreases BD7 to BD91 as transition from maternal to infant source
  – Juvenile Cynos similar immunoglobulins to adults
Teratologic Measurements

- **Skeletal**
  - Typically on BD28 (by digital radiograph)
  - Standard skeletal analysis also possible

- **External/Visceral**
  - Shape & symmetry of head and body
  - Morphometric measures
    - Crown-rump length
    - Tibia measurements
    - Head circumference
    - Anogenital distance
  - Internal organ morphology
  - Detailed heart evaluations
Specialised Skeletal Evaluation Techniques

- **Bone Length**
  - External measure
  - X-ray

- **Bone Densitometry (BMC and BMD)**
  - Dual energy X-ray absorptiometry (DXA)
  - Peripheral quantitative computed tomography (pQCT)

- **Bone Strength**
  - Biomechanical testing
  - Architecture (histomorphometry)
Advanced Skeleton Assessments

• Bone Quality
  – Bone mineral content (BMC)
  – Bone mineral density (BMD)
  – Measure BMC and BMD in vivo with,
    • Dual-energy X-ray (DXA) absorptiometry
    • Peripheral quantitative computed tomography (pQCT)
  – Architecture (histomorphometry)
  – Biomechanical strength testing

• Bone Growth Dynamics
  – Histomorphometry (fluorochrome labeling)
    • Bone formation rates (BFR), mineral apposition rates (MAR)
  – Biochemical markers of bone turnover
HISTOPATHOLOGY

“CHILD” ........ 12-35 MONTHS OLD

“JUVENILE” .... 36-48 MONTHS OLD
Histopathology: CRL HCD paper 2010

- HCD Cynos (12-36 mo); *Tox Path*, 38(4), 642-657
- Housed in groups of two or three animals per cage
- All serologically negative for:
  - Simian immunodeficiency virus (SIV)
  - Cercopithecine herpesvirus 1 (B virus)
  - Simian retroviruses type D
  - Rabies
  - Simian T-cell leukemia virus (STLV)
  - Measles
  - Filoviruses
- Tuberculin tests; screening tests for *Shigella*, *Yersinia*, and *Salmonella*; parasitological screening (including malaria smears) carried out on arrival at test facility
Most common findings

• Inflammatory cell infiltrates mononuclear/lymphoplasmacytic:
  – in liver (60%),
  – kidney (32.8%),
  – heart (25.2%),
  – salivary glands (23%)
  – stomach (11.7%)

• Inflammation:
  – kidneys (interstitial nephritis),
  – stomach (chronic gastritis),
  – heart & salivary glands

• Ectopic tissue: e.g. thyroid
Ectopic tissue (Congenital lesions)

- Epithelial and squamous cysts/plaques
- Thyroid gland (thyroglossal duct)
- Adrenal
Inflammatory cell Infiltrates

- Minimal-mild lymphoplasmacytic & minimal-moderate focal myocarditis
- 25-100% animals affected
- Uniform, aggregates with little/no associated damage to the cardiac myocytes
Myocarditis

- Infiltrates may progress to myocarditis: necrosis, karyomegaly, haemorrhage, fibrin deposition, lymphoplasmacytic cells, granulocytes and macrophages
- Subendocardial or subepicardial/epicardial, apex & base
- Simultaneous myocarditis and focal inflammatory cell infiltrates in same heart
Cardiomyopathy

- Idiopathic myocardial degeneration (cardiomyopathy)
  - Minimal-moderate localized/extensive degeneration or necrosis
  - Myofiber vacuolation
  - Mild-marked hypertrophy
  - Minimal inflammation
  - Karyomegaly
  - Fibrosis
Vascular lesions

- Perivasculitis: LI, meninges, lungs, sciatic n., urinary bladder
- Intimal thickening; formation of arteriosclerotic plaque; aorta, subendocardial heart base, coronary arteries $\rightarrow$ occlusion
- Accumulation of mucopolysaccharides in subendothelial aorta $\rightarrow$ disruption of IEL
- Ectopic thyroid
Lungs

- Focal pleural/ subpleural fibrosis & pleuritic
- Alveolar macrophage accumulation
- Perivascular or peribronchial inflammatory cells
- Focal interstitial inflammation
- Osseous metaplasia
Liver

- Mononuclear cell infiltrates or inflammatory cell foci within parenchyma or in the periportal areas
- Diffuse hepatocyte vacuolation
- Bile duct hyperplasia/fibrosis to mineralisation
- Extramedullary haematopoiesis
- Focal and/or single cell necrosis
Liver

• Capsular Fibrosis
  – Focal or localised; parietal surface in contact with diaphragm & costal wall; visible at necropsy
  – Associated with boisterous playing or aggressive behaviour
  – May occur in association with tension lipidosis
  – May be adhesions to diaphragm
Central Nervous System

• Perivascular inflammatory cell infiltrates within meninges of the brain and spinal cord
• Perivascular lymphocyte cuffs within the cerebellar peduncle, periventricular areas & around the vessels of the choroid plexus.
Brain

- Gliosis with neuronal necrosis & neuronophagia
- Glial scars with pigmented microglial cells & some small spheroid bodies
- Macaques susceptible to gliosis and/or necrotic neurons after minor head injuries caused by fighting with cage mates or from banging into perches/cage sides when playing
- Occasionally seen after recent anaesthesia (volatile agents)
Lymphoid system

- Extramedullary hematopoiesis in cutaneous draining nodes (local to injection sites)
- Pigment deposits in LN
- Granulocytic infiltrates in submandibular LN
- Histiocytosis in mesenteric LN
- Follicular hyperplasia in LN
- GALT/BALT hyperplasia
Adrenal

- Ectopic adrenal in liver, kidney & male reproductive organs
- Extramedullary haematopoiesis
- Cortical vacuolation
- Focal mineralization at corticomedullary junction caused by dystrophic calcification of remnants of the fetal cortex
Adreno-hepatic fusion (AHF)

- Fusion between liver and right adrenal
- More common than adreno-hepatic adhesion (distinguished by the lack of fibrous tissue between the two parenchymal tissues)
- Normal appearing hepatocytes located between medulla & zona reticularis, & occasionally in cortex or hilar region of right adrenal gland
Intramuscular injections

Alum granulomas after vaccination
Conclusions: NHP Juvenile Toxicity Testing

• NHPs are not the recommended species for juvenile toxicity testing, but may be used when scientifically justified
• NHP can be optimal model to support biologics (pharmacology, immunogenicity)
• Can add juvenile endpoints to PPN Study
• Relatively large number of studies have now been conducted (historical control data)
• Regulatory understanding of design/interpretation has evolved
• Current designs emphasize 3Rs via study consolidation
References


• FDA (2006) Guidance for industry: considerations for developmental toxicity studies for preventative and therapeutic vaccines for infectious disease indications

• Chamanza R, Marxfeld HA, Blanco AI, Naylor SW, Bradley AE (2010) Incidences and Range of Spontaneous Findings in Control Cynomolgus Monkeys (Macaca fascicularis) used in Toxicity Studies Tox Path, 38(4), 642-657
Any Questions?