Discussion of external, visceral and skeletal anomalies in nonhuman primates regarding their integration into new terminology and classification

7th Workshop on the Terminology in Developmental Toxicology

4. – 6. May 2011

Berlin

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Overview

- Type of studies in NHP
- Conclusion after review of new terminology
- Differences between rodents/rabbits and NHP
- Frequent and rare findings in NHP not found in the new terminolgy list
- Examples where we had a problem to include a finding to the list
- Enhanced pre- and post-natal studies and use of terminology
- Classification of findings
- Conclusion

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Primate species used in EFD/ePPND studies

Cynomolgus monkey (M. fascicularis)

- Established nonhuman primate model

Common Marmoset (Callithrix jacchus)

- Metabolic profile of test article
- Specific cross-reactivity (biologics)
- Reacts to thalidomide derivatives
  - Neubert; Hendrickx

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Embryo-fetal Development Study in the Cynomolgus Monkey

Mating

Pregnancy
- confirmation by ultrasound

Mother
- maternal blood
- clinical pathology
- immunophenotyping
- immunoglobulins

Fetus
- cardiac assessment
- morphometry

Cesarean section
- fetal examination
- organ weights
- lymphatic organs
- histopathology
- CD immunohistochemistry
- maternal/fetal IGs
- morphometry
- amniotic fluid
- lymphocyte proliferation
- umbilical cord blood (flow)

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gestational day														
36	44	46	48	49	51	53	57	60	65	68	85	100	120	150
length, cm (coccyx-cranium)														
1,3	2,0	2,5	2,8	3,1	3,6	4,1	4,6	5,3	5,9	6,1	8,2	12,3	14,6	17,1

Embryo-fetal Development Study in the Marmoset Monkey

After review of new document:

- In general, terminology for fetal examination can be used for primates
- Several special findings or regions need to be added

Advantages of use of terminology:

- Harmonized wording within and across labs
- No strange descriptions or translations of German findings
- Easier for staff involved in examination and data evaluation
- Easier to compare findings in rodent/rabbit studies with those in primate studies for clients and reviewers

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Differences to rodents/rabbits

Main differences to rodents/rabbits

- Single uterus
- Split placenta in Cynos
- Single fetus in Cynos, max. twins
- One to four fetuses in Marmosets
- Seven sternebrae – use of new list is no problem
- More skull bones at skull basis
- At GD 150 ossification of tarsal and carpal bones
- Monkeys have hands, feet, fingers and toes, not paws and claws
- Penial bone in male Cyno fetuses

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Review of reference data and current studies for:

- Frequent and rare findings in NHP not found in the new terminology list
- Example where we had a problem to include a finding to the list

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Maternal-fetal findings

Umbilical cord –

- only two vessels (normal case is three vessels)
- edematous
- swollen
- red discolored
- twisted
- one artery, one vein
- not visible, dry (after delivery prior to c`section scheduled for GD 150)

Placenta -

- focus (could be reported as discolored)
- 2nd part discolored, swollen

Maternal-fetal

Placenta, cynomolgus monkey, GD 100

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External findings

- **Prepuce not or incompletely patent**
- Genital region – enlarged
- Craniofacial region - amniotic banding
- Anterior vulva region – reddened
- Ear/s - additional tissue flag on lobule of auricle
- Sternum - bent outwards/inwards
- Thorax – misshapen
- External genitals – absent, reduced in size, swollen, red discoloured
- **Head – poor hair**
- **Fur findings in general for fetuses collected GD 150**
- Tail constrictions, incisures, flattened
- **Foot joint misshapen**
- **Abnormal position of fingers/toes**
- **Finger/toe nail - reduced in size, absent**

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External, skeletal

- Monkeys have hands, feet, fingers and toes, not paws and claws

Fetal „forepaw“

External findings

- Additional nipples (three or four)
- Rare variation, also observed in adults

External findings

- Small tissue ball at tail end
  - most common external finding
  - normally no correlating skeletal finding
  - variation
  - lost after birth
- Now included in the terminology as fleshy tab

Visceral findings

- Adrenals – soft consistency
- Amnion fused to cerebral hemispheres and cerebellum
- Cerebellum hypoplastic
- Eyes – discharge of liquid
- Heart – hematoma at apex
- Spleen – firm consistency
- Testes – blood shot

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Visceral findings

Stomach

- foci at cardia
- foci at cardia region
- foci at fundus
- hemorrhage at mucosa
- hemorrhage at cardia
- hemorrhage at cardia region
- reddened cardia
- hemorrhage
- hemorrhage at fundus
- hemorrhage at pylorus

Could all be reported as

Stomach wall – discolored

But:

-reference data
-detailed findings necessary due to low number of fetuses

A more uniform, but detailed terminology useful – grouping of findings for the report

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Visceral findings

Further examples:

Thymus:

- Multiple small hemorrhages
- Small red spots
- Red focus
- Reddened
- Red spotted
- Bright red patterned
- Red patterned
- Black-red discolored

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Visceral findings

Structures not included in the new terminology:

- Salivary glands
- Lymphnodes

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Skeletal findings

Skull, base, GD 100, to be examined:

- Ala minor ossis sphenoidale
- Ala major ossis sphenoidale
- Os frontale
- Os parietale
- Os occipitale
- Pars lateralis ossis occipitale
- Foramen magnum
- Clivus
- Os petrosum
- Sella turcica
- Os temporale

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Skeletal findings

- At GD 150 ossification of tarsal and carpal bones:

Hand:

- Trapezium
- Triquetrum
- Scaphoideum
- Capitatum
- Hamatum
- Lunatum
- Pisiforme

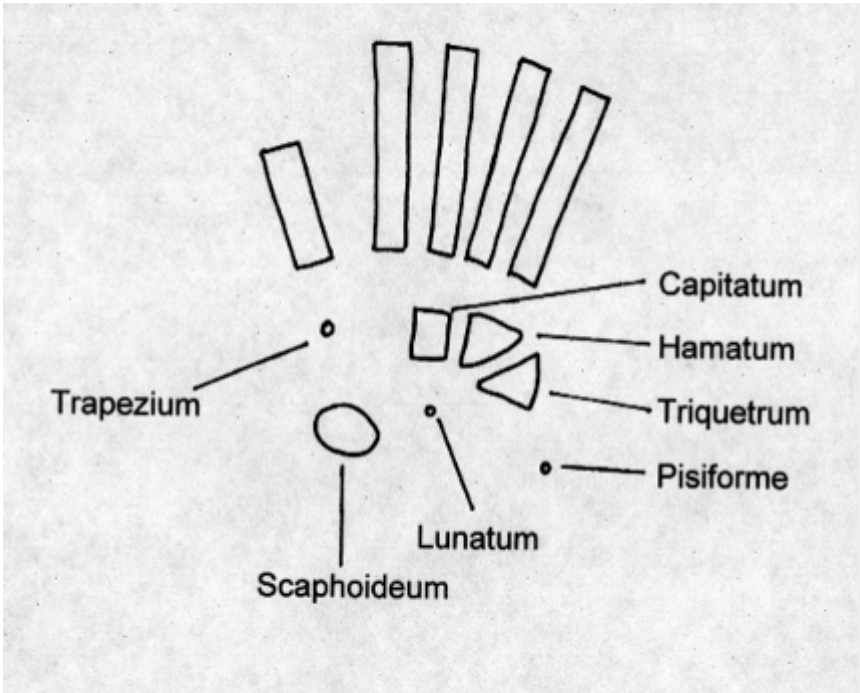
Marmosets: central bone

Foot:

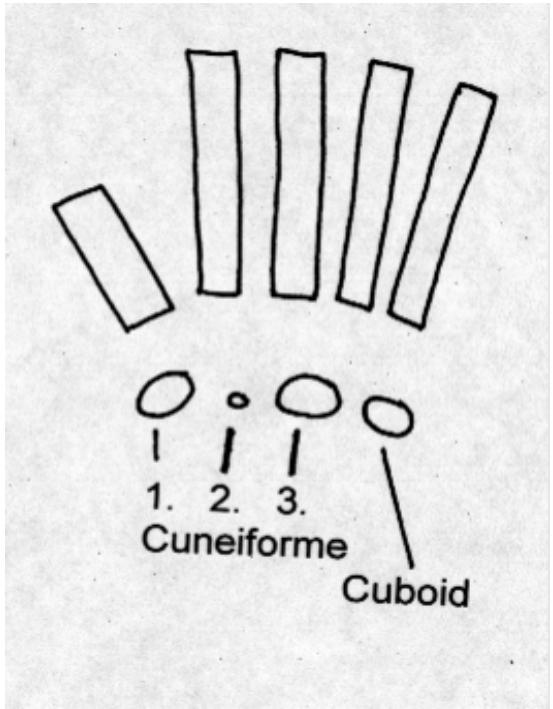
- Cuneiforme 1 to 3
- Cuboideum
- Navicular

Skeletal findings

Hand, normal ossification GD 150



Foot, normal ossification GD 150



Skeletal findings

NHP (Covance) specific findings (?):

- Additional ossification site prior to normal 1st cervical vertebra, may be bipartite
- Last vertebra called zygostyle
- Bones of tail historically named coccygeal vertebrae, will now be changed to caudal
- Penial bone present, can be incompletely, not, asymmetrically ossified, bent etc. (Cynomolgus)

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Skeletal findings

Recently found:

- irregular ossification – irregular, clear line, looks different from incomplete ossification

Ribs:

- suture like site – Marmoset infant day 29 p.p.; fracture of the ribs? Unclear.

Costal cartilage process

- proximally fused, then branched
- branched, articulation to …sternebrae
- branched, reunited at sternum - ringshaped

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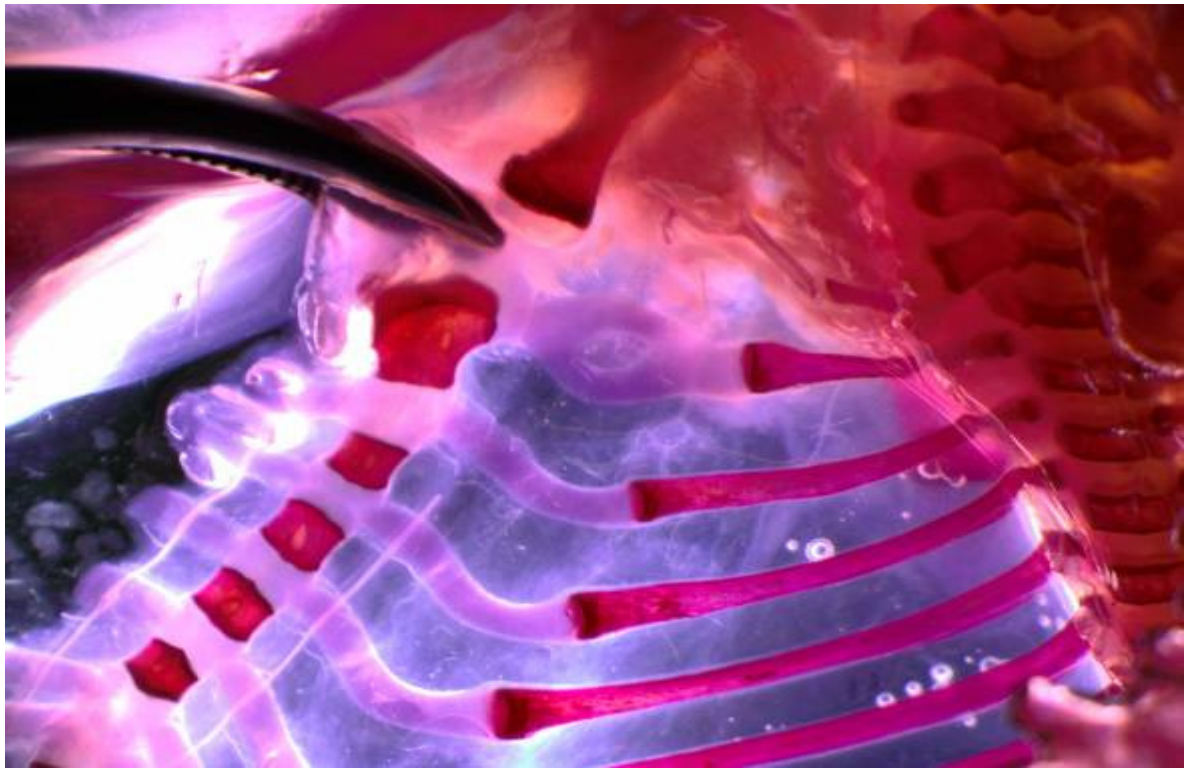
Skeletal findings

- Costal cartilage process branched, reunited at sternum – ringshaped (malformation or variation?)



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Skeletal findings

Sternum

- bent inside, outside

Pelvic girdle

- Hole
- Insertion on last lumbar and first sacral vertebra
- Insertion on first and second sacral vertebra

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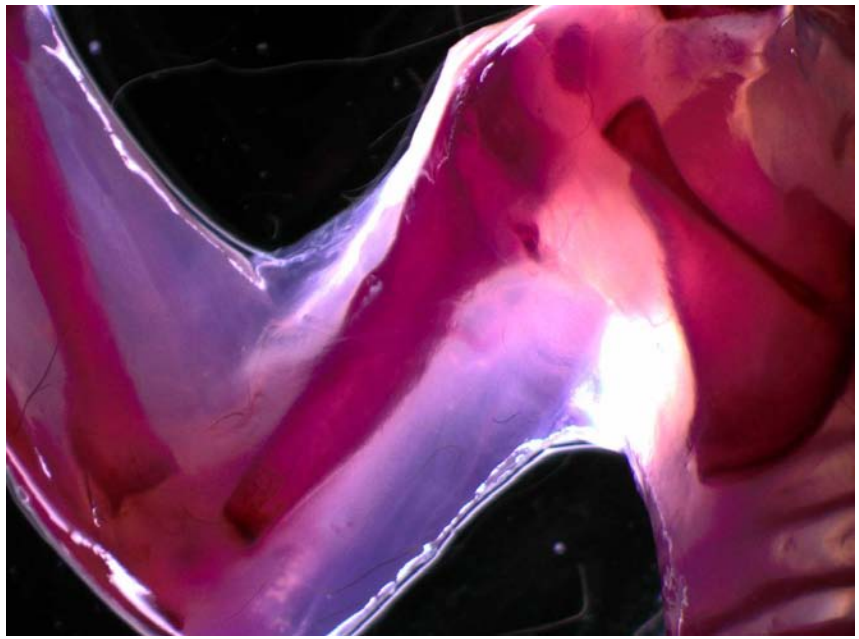
Problems with use of the list

- Cynomolgus, GD 100 - Wide and severe red coloration of the skin

- Marmoset, Day 29 p.p.: Humerus right, irregular shape or ossification? Malformation vs variation?



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ePPND Studies

- For biologics, only an enhanced pre- and post-natal study is required.
- ➔ embryonic-fetal development check by ultrasound, no cesarean section, external examination of newborns, x-rays of infants at one to three months of age and necropsy at the end of the study
- Use of new terminology for this type of studies difficult but should be considered as far as possible

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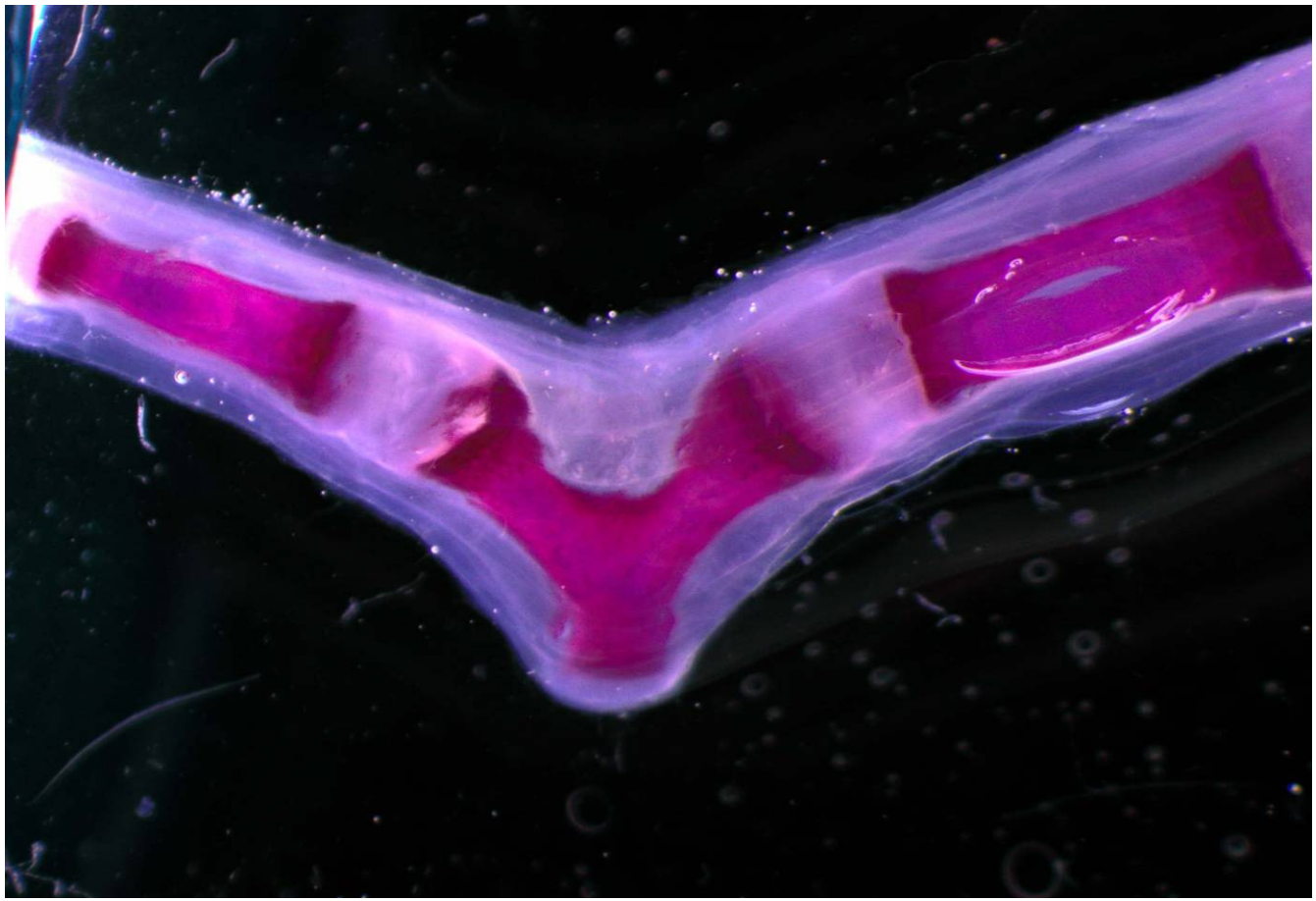
Weakness of x-ray

- Marmoset, day 29 p.p., misshapen caudal vertebra



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Classification of findings

Classification of findings into variation and malformation is regularly requested by clients, auditors or reviewers

A general, published classification

- Would lead to harmonized classification in all labs
- Would help for discussion of findings with clients and reviewers
- Would avoid discrepancies between labs
- Would avoid discrepancies also within a lab when different study directors need to classify findings for different studies
- Would help staff involved in data evaluation

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Classification of findings

Different classification of a finding would need explanation by the study director

Differences between species have to be considered

- more than two nipples - normal in non-primates, variation in primates;
- penial bone absent - malformation in cynomolgus monkeys, normal in marmosets
- Cervical ribs - ???

Conclusion

- Use of new terminology for DART studies in NHP (cynomolgus, marmoset) in general no problem
- For inclusion of NHP specific terms an appendix could be added
- Agreed terminology is essential for electronic data capture which is upcoming for NHP DART studies
- Special, unusual findings can be added if necessary, but do not need to be included into the general list
- Published classification of findings useful
- For ePPND studies, the use of the agreed terminology should be considered as far as possible

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