Non-Rodent Juvenile Toxicology

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Acknowledgements

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Presentation Outline

- General considerations
- Species selection and use
- Dog model
- Minipig model
- Nonhuman primate model
- Summary

Juvenile Toxicity Studies- When to do

- **Completed studies form basis for predicting risk to pediatric population**
  - Nonclinical data from adult animals
  - Nonclinical reproductive development studies
  - Clinical trials in human

- **If insufficient data to assess risk, juvenile toxicity study may be required.**

Key points in Juvenile Toxicity Studies

- **Understanding comparative organ development in test species vs. child**
- **Understanding PK/ADME profiles in test species vs. child**
- **Understanding logistical requirements**
  - Cross-fostering, reproductive capacity, etc.

Background info to design Juvenile Tox study

- Indication in pediatric population
- Mechanism of action
- Sensitivity of test species
- Justification of test species
- Target organ toxicity
- Route of administration
- Gap assessment of available repro/developmental studies
- Other data: TK, structural activity, etc.

Age Comparison Between Humans and Animal Models

Species	Preterm	Newborn	Infant	Child	Adolescent
Human	–	0–28d	1–23m	2–12y	12–16y
Monkey	–	0–15d	0.5–6m	0.5–3y	3–4y
Dog	–	0–21d	3–6w	6–20w	5–7m
Pig	–	0–15d	2–4w	4–14w	4–6m
Rabbit	0–4d	0–10d	1.5–5w	5–12w	3–6m
Rat	0–4d	0–10d	1.5–3w	3–6w	7–11w
Mouse	0–4d	0–10d	1.5–3w	3–5w	5–7w

Barrow et al (2011) Methods Mol Biol 691:17-35

EPL

Comparative Age Categories Based on CNS and Reproductive Development

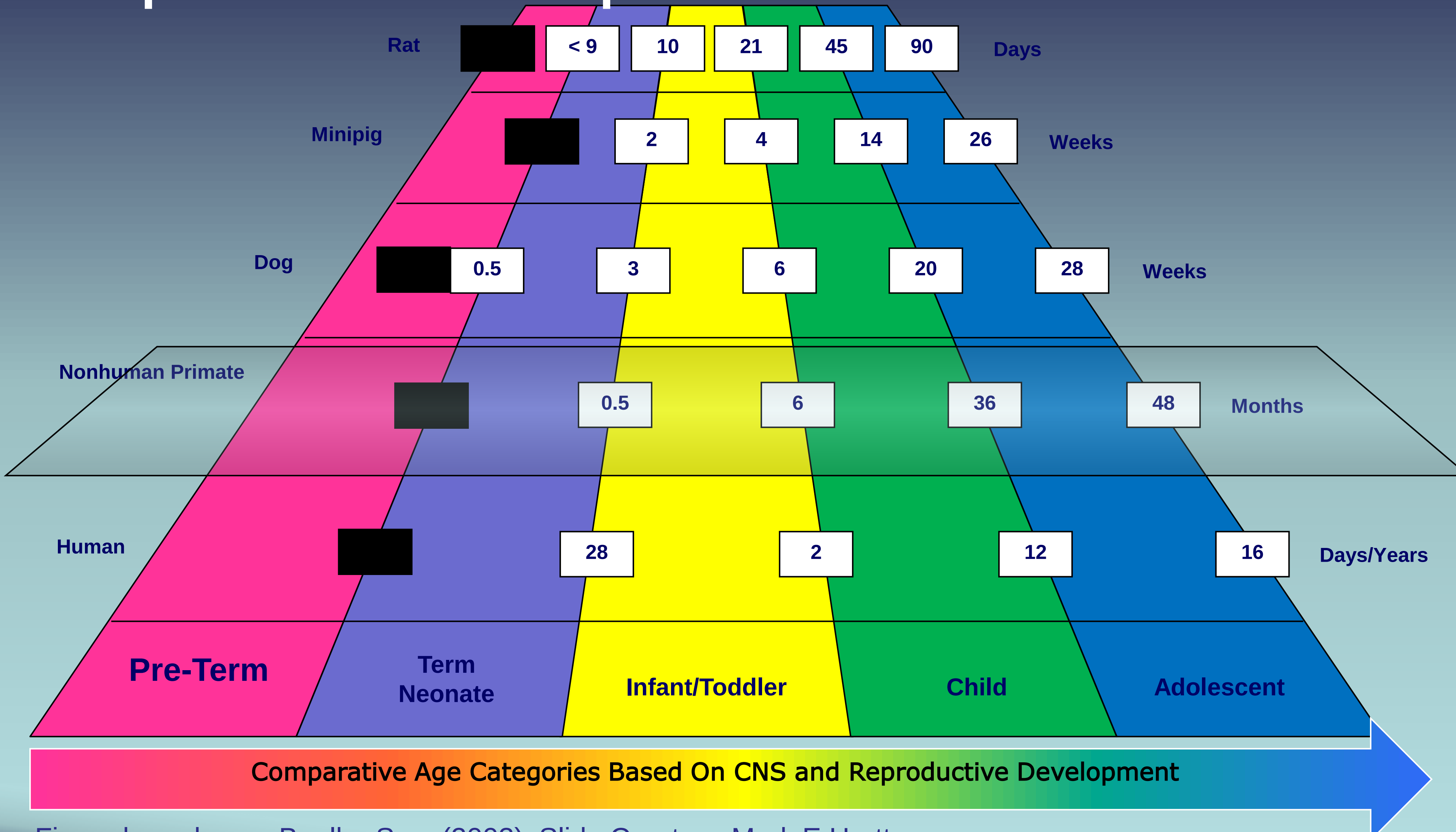
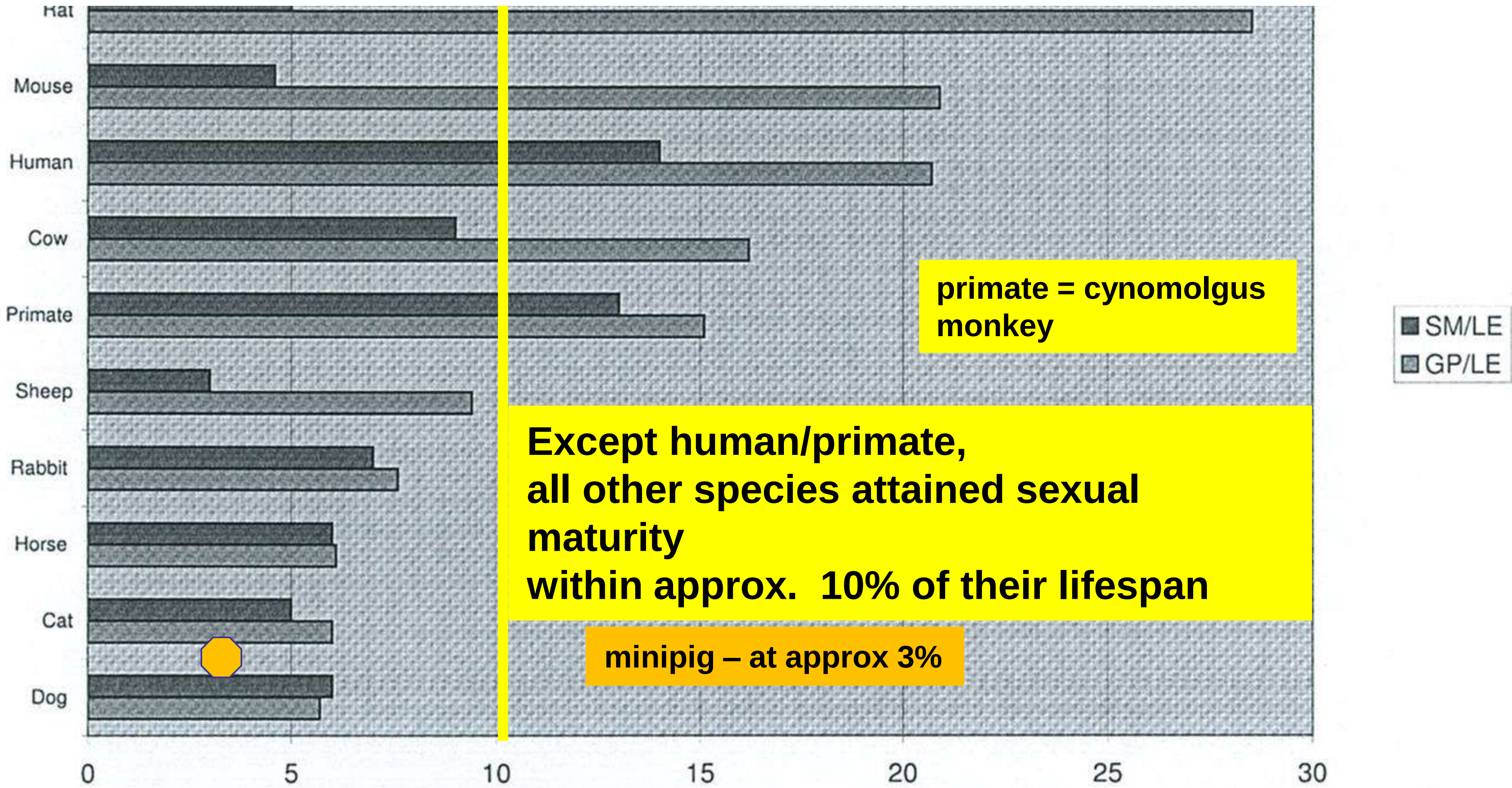


Figure based upon Buelke-Sam (2003), Slide Courtesy Mark E Hurtt

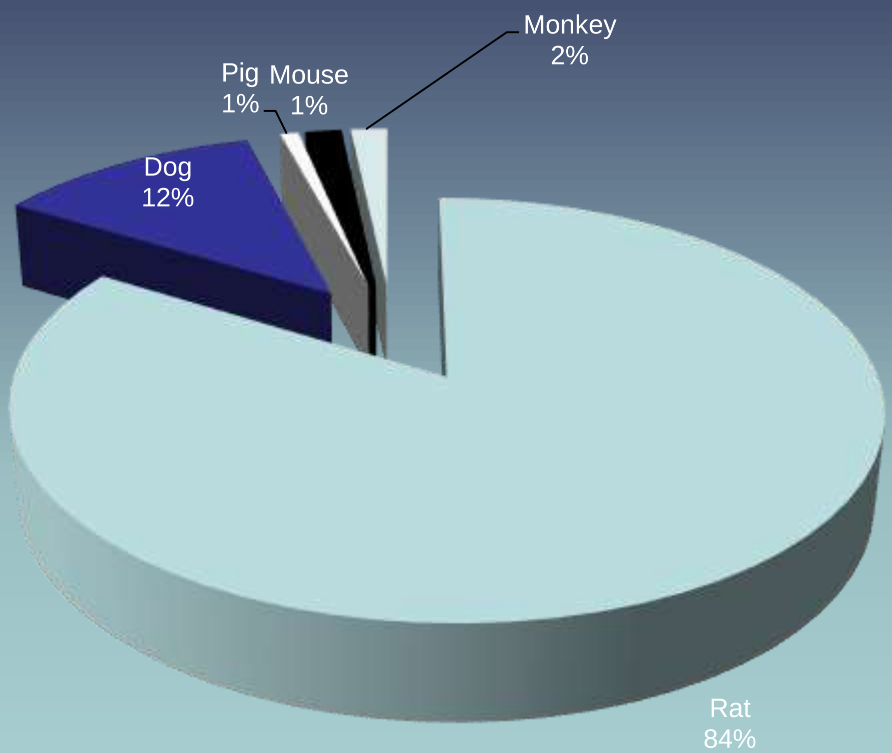
www.epl-inc.com

Ratio of Age at Sexual Maturity and Growth Plate Closure to Life Expectancy



SM = sexual maturity, LE = life expectancy, GP = growth plate closure
Kilborn et al (2002) Cont Topics 41: 21-26 (AALAS)

What Species is Most Popular?



- Industry survey:
- Of 241 studies from 24 companies, 202 used rat
- 29, 4, 4, 2, mention dog, mouse, monkey, minipig, respectively

Bailey & Marien (2011) Birth Defs Res (Part B) 92:273–291

Design Approaches

Target organ-specific case-by-case study designs

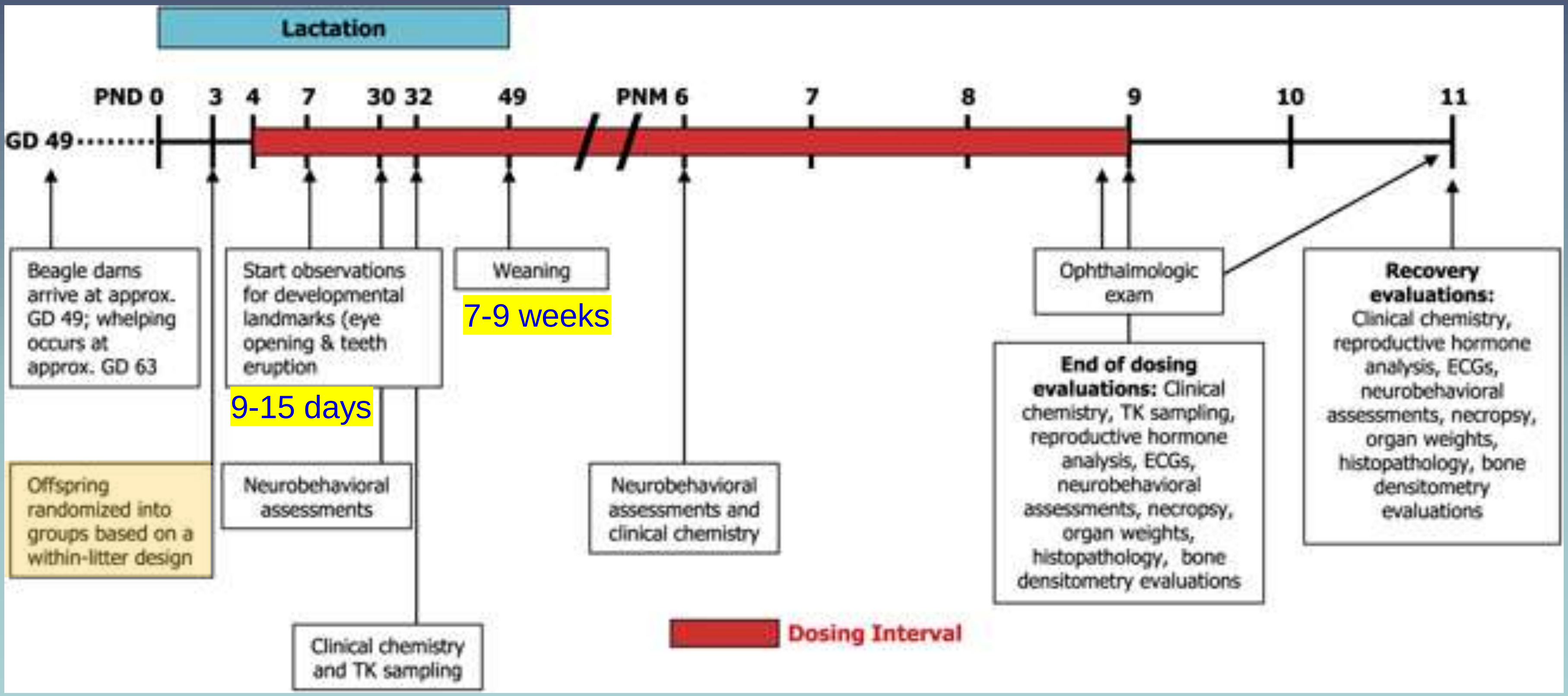
- Focused or targeted designs
- Detailed evaluation of specific organ of concern
- Comparable developmental processes across species is critical
  - Exposure period must be at same stage of development as humans

Generalized/screening toxicity study designs

- Similar to repeat-dose toxicity studies
- Dosing begins at younger age
- Covers broad periods of organ system development
- Evaluates same endpoints as standard toxicology studies
- Other endpoints are added as appropriate

Beagle Dog

Juvenile Dog Screening Study for CNS Active Compound



Cappon et al (2009) Birth Defs Res Part B 86:463-469

Dog Case Study – Vigabatrin (SABRIL®)

- Antiepileptic drug indicated for infantile spasms in children aged 1 month to 2 years
- Administration of vigabatrin to neonatal rats in a previous study was associated with changes in the gray matter of the brain and decreased myelination, which were considered distinct from findings in adult animals given this drug
- The objective of the study reported here was the assessment of neurohistopathological changes in the brain during an oral gavage study in juvenile (preweaned) beagle dogs

Bottomley et al (2015) Toxicol Pathol,

Dog Case Study – Study Design

Group	Treatment (mg/kg/day)	Study phase	Dosing days and nominal number of animals allocated					
			90 Day Study PND 22 to 112 (Weeks 4 to 15)		Intermediate I PND 22 to 35 (Weeks 4 + 5)		Intermediate II PND 36 to 49 (Weeks 6 + 7)	
			Number	Day*	Number	Day*	Number	Day*
1	0	main recovery	6 6	113 155	7 5	36 50	4 5	50 64
2	Low	main recovery	4 4	113 155	5 4	36 50	4 4	50 64
3	High	main recovery	4 4	113 155	5 4	36 50	5 4	50 64

* First day of necropsy

(Courtesy Anna Bottomley)

Dog Case Study – Group Allocation

		90 day Study			Intermediate I Study			Intermediate II Study		
Litter	Available pups	Controls	Low	High	Controls	Low	High	Controls	Low	High
A	3M + 5F	1M + 2F	1M + 2F	1M + 1F						
B	1M + 7F	1M + 2F	2F	3F						
C	4M + 4F	1M + 1F	2M + 1F	1M + 2F						
D	4M + 3F	3M + 1F			1M + 2F					
E	4M + 2F				2M	1M + 1F	1M + 1F			
F	3M + 2F				1M + 1F	1M + 1F	1M			
G	2M + 7F				1M + 2F	2F	1M + 3F			
H	4M + 2F				1M	2M + 1F	1M + 1F			
I	4F							4F		
J	3M + 4F							1M	1M + 2F	1M + 2F
K	4M + 5F							1M + 2F	1M + 2F	2M + 1F
L	5M + 1F							1M	2M	2M + 1F
	Total / group	6M + 6F	3M + 5F	2M + 6F	6M + 5F	4M + 5F	4M + 5F	3M + 6F	4M + 4F	5M + 4F

(Courtesy Anna Bottomley)

Dog Case Study – Postnatal Day 1 to 21

- Puppies weighed daily and litter observations performed twice daily
- Parental bitches gradually acclimatised to 2-hour separation from their offspring so that, once dosing commenced, the puppies could be kept away from dam for 1 hour before dosing and up to 1 hour after
- Puppies initially identified by natural coat markings and then from two weeks of age by microchip

(Courtesy Anna Bottomley)

Dog Case Study – Weaning

- Lactation can be very demanding on the dam; they consume up to 3–4 times their normal daily amount of food when milk production reaches its peak at 3–4 weeks postpartum
- At approximately Day 40 postpartum all pups were offered supplementary ‘Lamlac milk’
- The parental bitches ‘time away from litters’ into a separate unit was gradually extended from Day 55 postpartum

(Courtesy Anna Bottomley)

Juvenile Dog Model

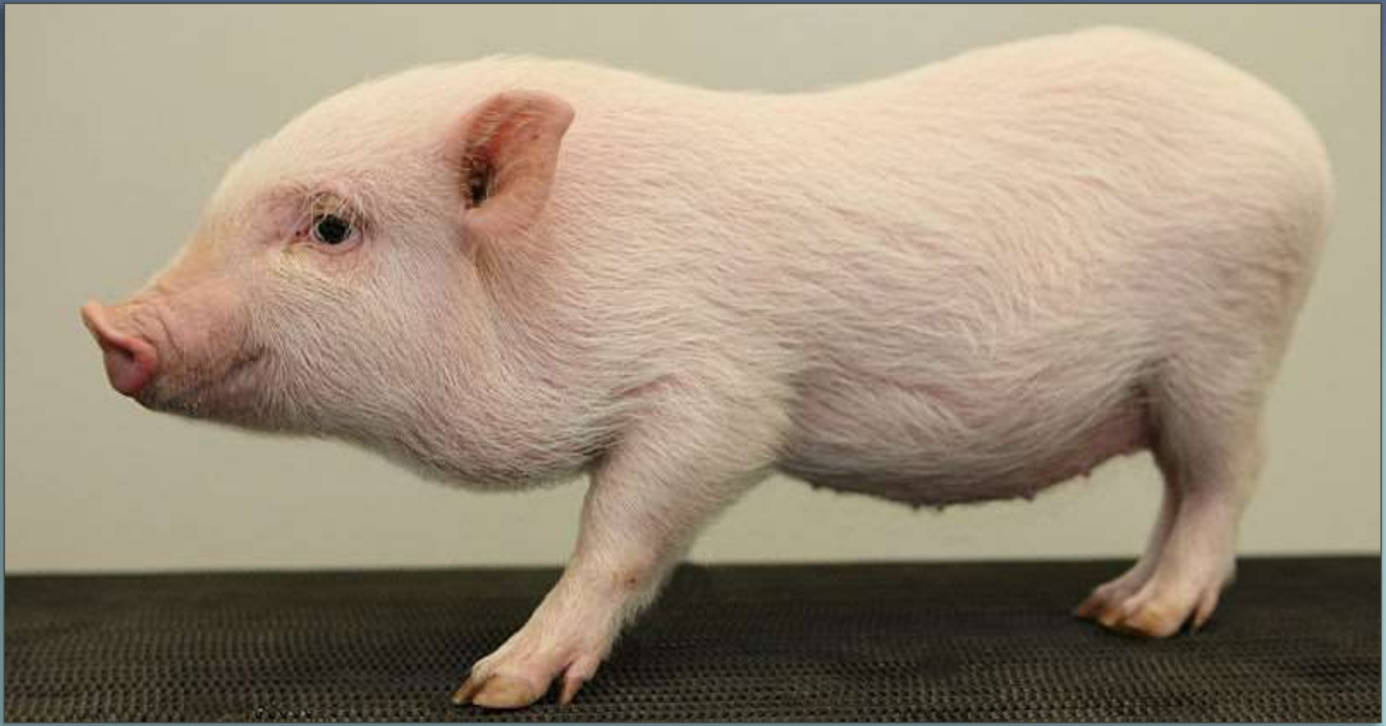
Advantages

- Provides model of immaturity
- Postnatal development of many organ systems well characterized
- Physical size facilitates collection of multiple biologic specimens
- Samples can be collected with minimum restraint and without anesthesia
- Techniques for handling and treating puppies well established
- Ability to procure appropriate numbers of animals, even for early-age assessments

Disadvantages

- Long time to sexual maturity limits assessment of reproductive development
- Limited reference or historical control data for some endpoints
- Learning and memory tests not well developed
- Potential immunogenic response
- Pharmacological relevance may not have been previously characterized
- Statistical analysis

Gö(oe)ttingen Minipig

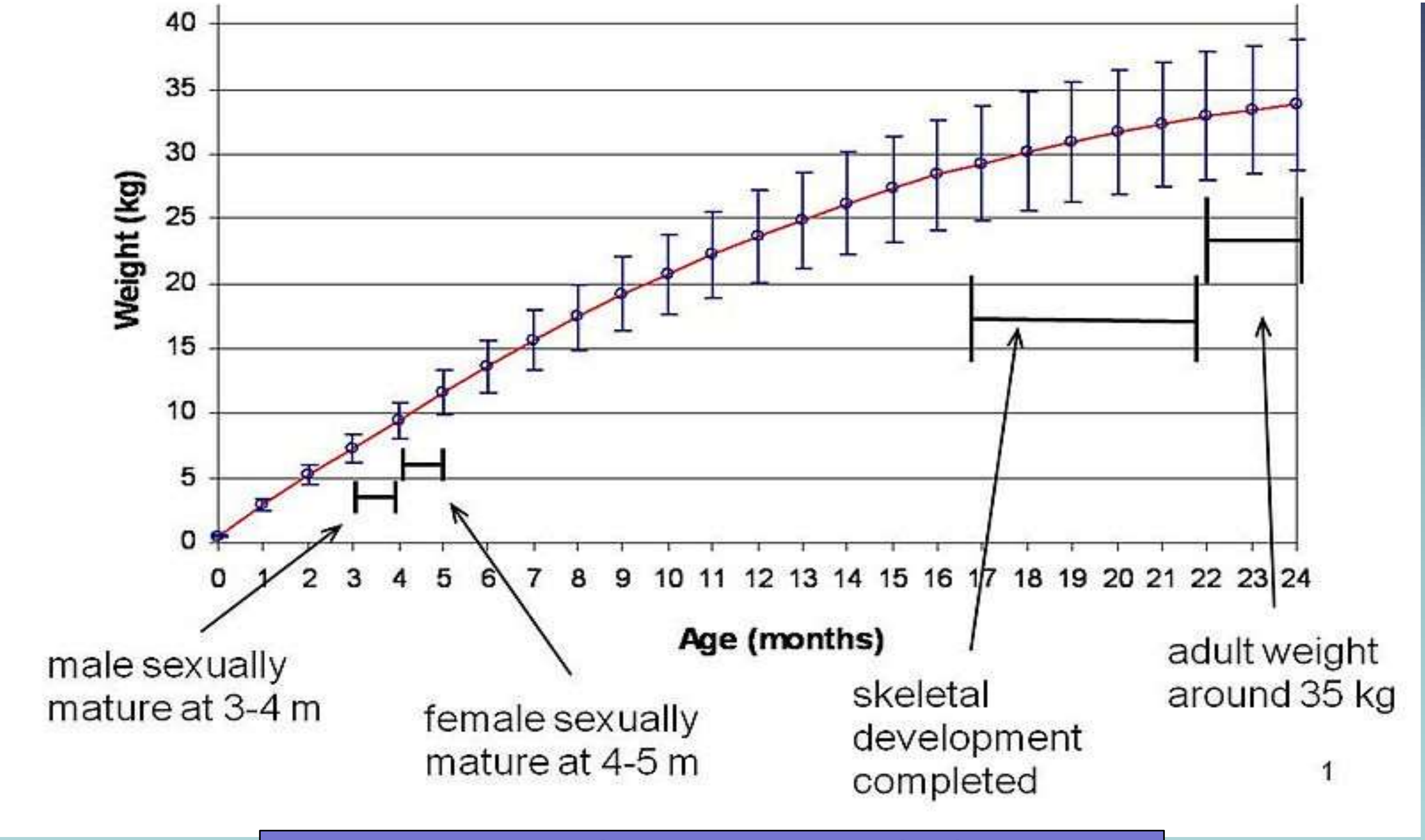


Minipig Juvenile Toxicity Model

Easier to work with than preweaned dogs:

- Pregnant sows easier to obtain
- Pregnant sows easier to ultrasound scan to determine litter size *in utero*
- Litter size (~6) more consistent
- Piglets can readily be cross-fostered
- Temporary removal from the mother is better tolerated
- Piglets wean at 4–5 weeks, puppies at 8 weeks

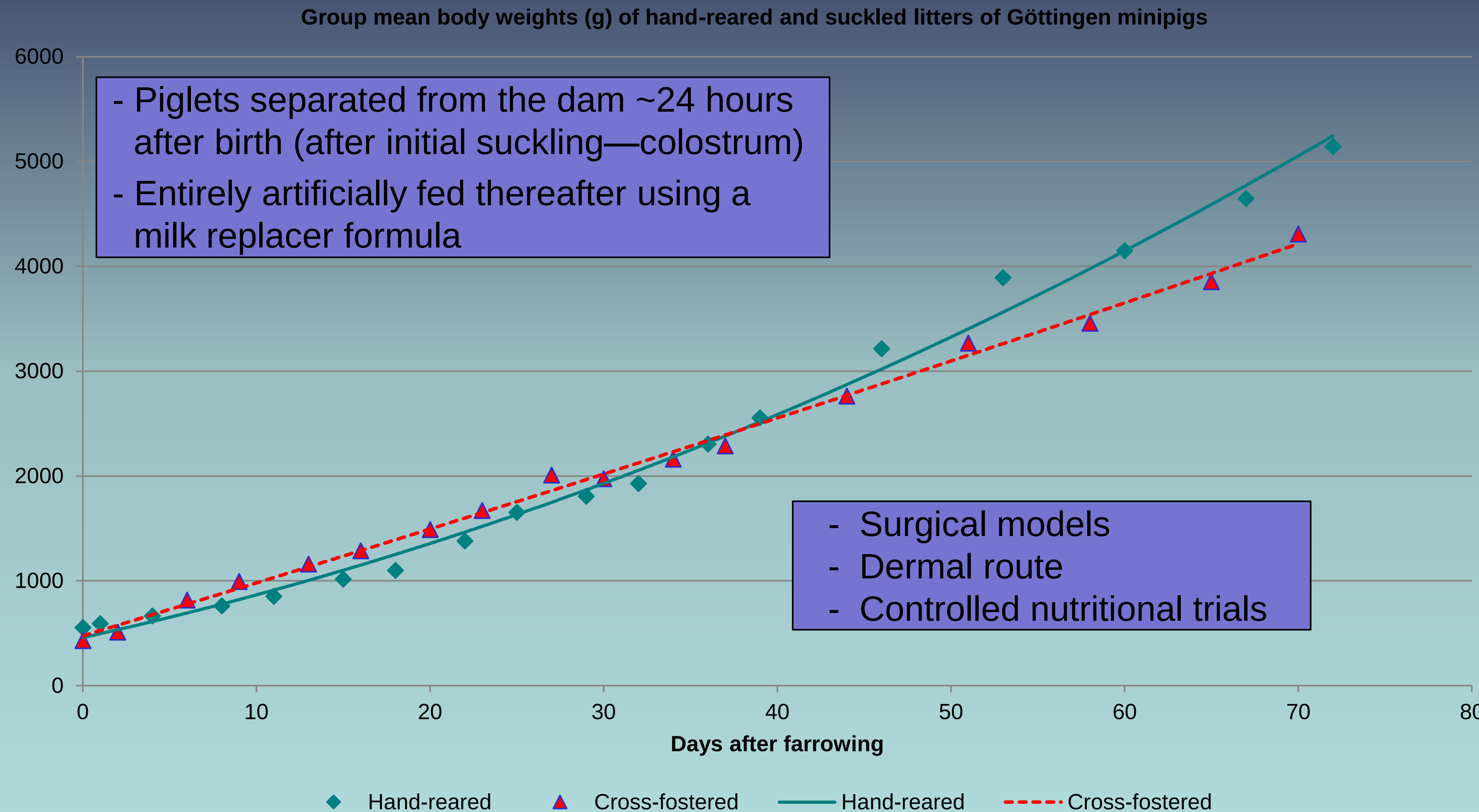
Growth Curve (Göttingen)



Female sexual maturity at 6.5–7.5 months (Tortereau *et. al.* (2013) *Toxicol Pathol* 41(8):1116-25)

Source: Wikipedia.org

Bottle-Raised vs. Suckling Piglets – Body Weight

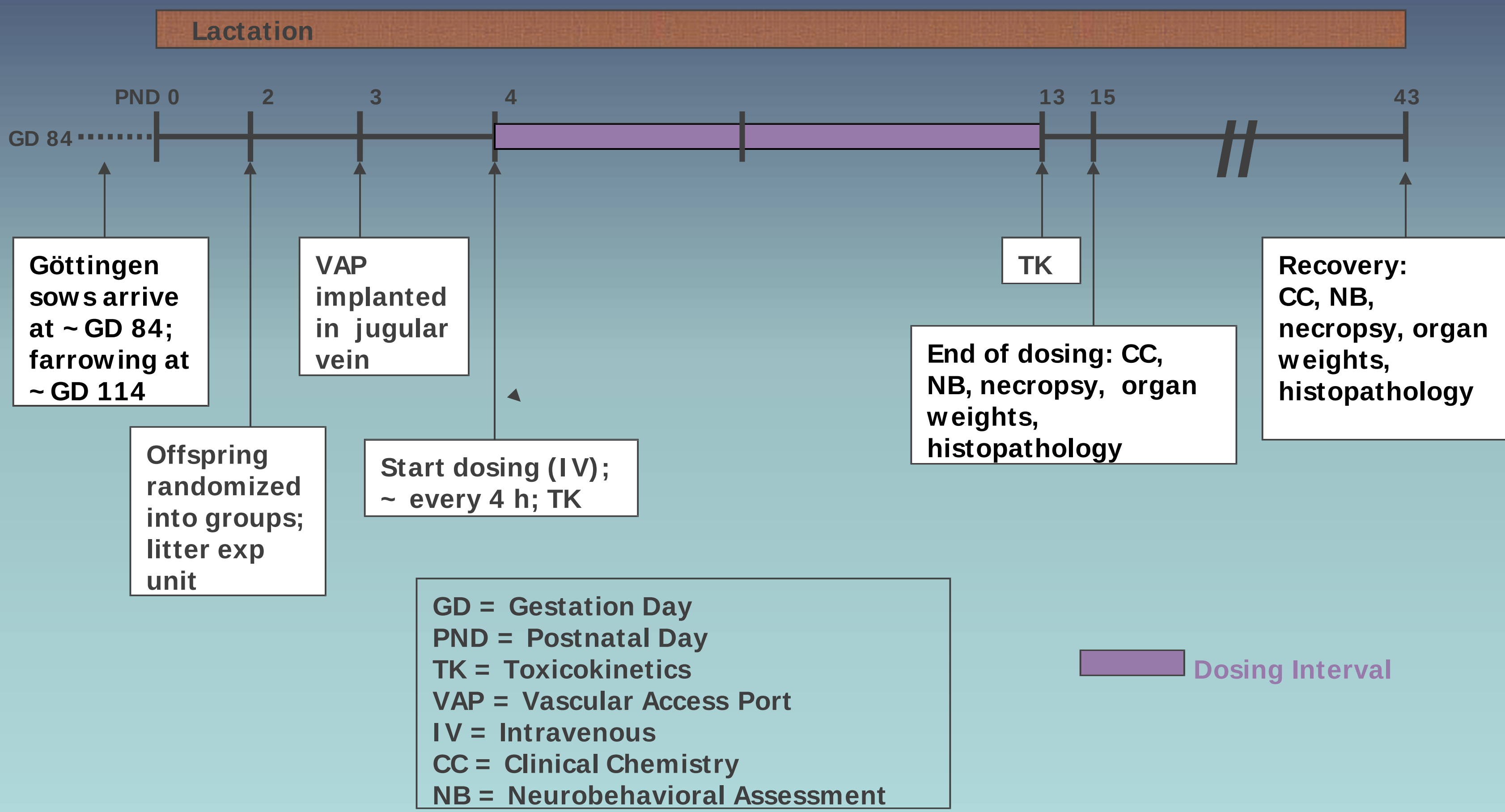


- Piglets separated from the dam ~24 hours after birth (after initial suckling—colostrum)
- Entirely artificially fed thereafter using a milk replacer formula

- Surgical models
- Dermal route
- Controlled nutritional trials

(courtesy Vanessa Ross)

Juvenile Toxicity Study in Minipigs Outline



Melissa Beck (Ellegaard website; http://www.minipigs.dk/)

Juvenile Minipig Model

Advantages

- High pregnancy rate and synchronization of mating possible
- Gestation length shorter than NHP
- Similarity of organ system anatomy, size and physiology to humans
- Availability of reference data for juvenile organ development
- Piglets are easily accessible for handling and dosing and allow frequent blood sampling
- Sexual maturity at 4–5 months (??)

Disadvantages

- Limited pharmacological relevance
- Neurobehavioral tests not well developed
- Potential immunogenic response
- Limited labs available with experience
- Sexual maturity at 6.5-7.5 months ?? (Tortereau *et. al.* (2013) *Toxicol Pathol* 41(8):1116-25)

Nonhuman Primates
Juvenile vs. Neonatal Animals

Postnatal Timing of Selected Organ System Developments/Recommended Minimum Ages

Organ system	Cynomolgus	Human	Recommended minimum study age
Skeletal**			
Ossification centers	0–6 months	6 months–12 years	12 months
Epiphyseal closure	5–9 years	11–20 years	not applicable
Immune			
Spleen/lymph node	Birth	Birth	not applicable
Immunocompetence	0–3 months	0–3 months	3 months
Reproductive	3–6 years	8–14 years	not applicable
Cardiovascular	Birth	Birth	not applicable
Renal	Birth	12 months	12 months
(Central) Nervous			
Locomotion	7 weeks	9–13 months	2 months
Fine motor/dexterity	6 months	1.5–13 years	6 months
Sensory/reflexes	0–12 months	0–12 months	12 months
Cognition	3 weeks–3 years	1 months–9 years	3 years
Communication	0–12 months	0–24 months	12 months
**Pulmonary	Birth	2–8 years	not applicable
Digestive	0–8 months	0–24 months	8 months

Based upon Table 1 from Martin & Weinbauer (2010). The right column represents the recommendation for the minimal age of macaques for studying potential organ system effects. ** denotes systems with major functional differences compared to human postnatal development. Bone age at birth is far more advanced in macaques than in humans. For the skeletal system, differences also depend on which bone is being studied.

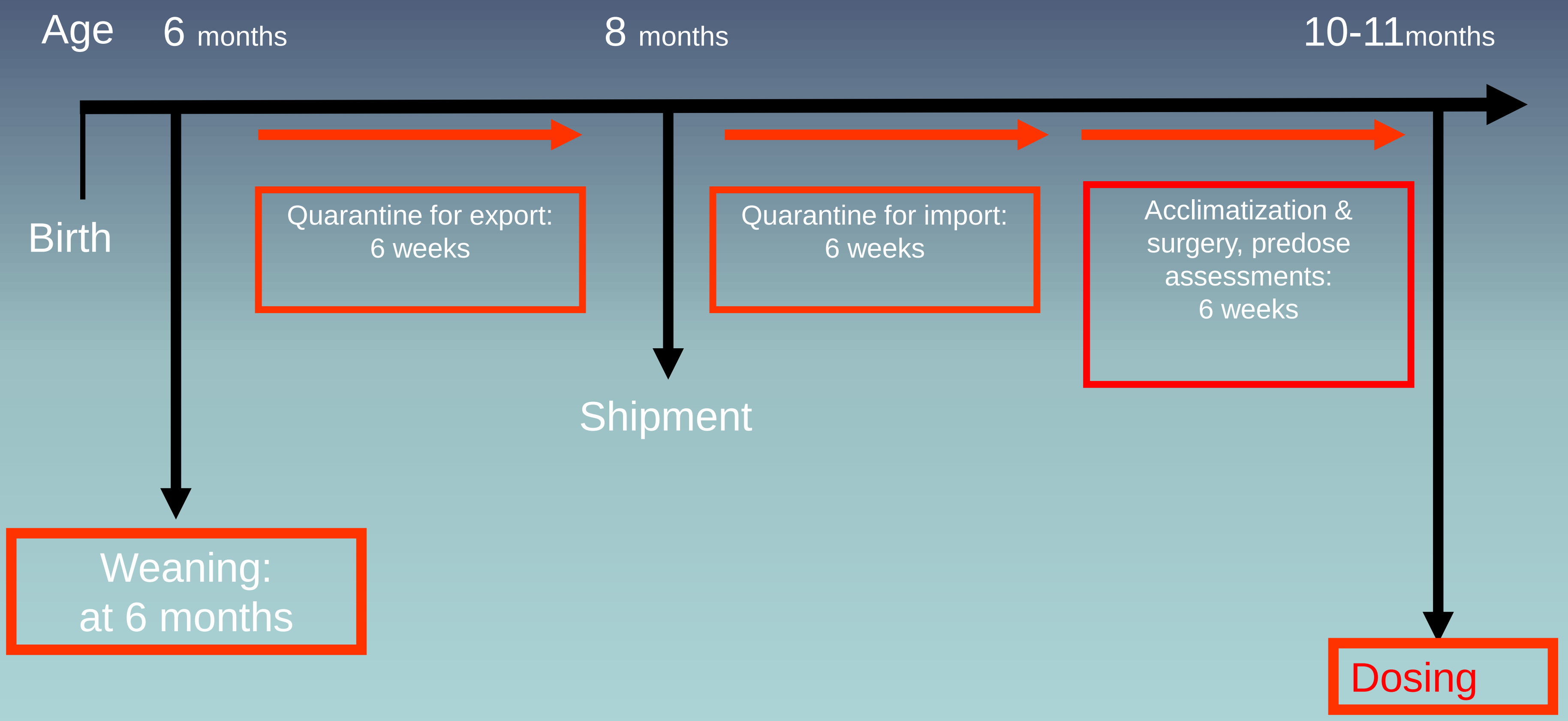
Morford et al (2011) Birth Defs Res Part B 92:359-380

Animal Supply Considerations: Neonatal vs. Juvenile

- Time-mated NHPs are not available from animal suppliers
- Import infants without mothers
  - Animals may need to be older than > 12 months (supplier differences)
  - Occasionally 9–12 months acceptable
  - Typical approach to NHP juvenile toxicity evaluation
- Import infants with mothers (approx. 5.5 months of age)
  - Caveats:
    - difficult supply
    - further use of maternal animals?
- Generate neonatal/juvenile animals
  - Start with mature females, mating, pregnancy and postnatal development, takes approx. 16 month,
  - Caveats:
    - pre-/postnatal loss
    - no control of gender distribution

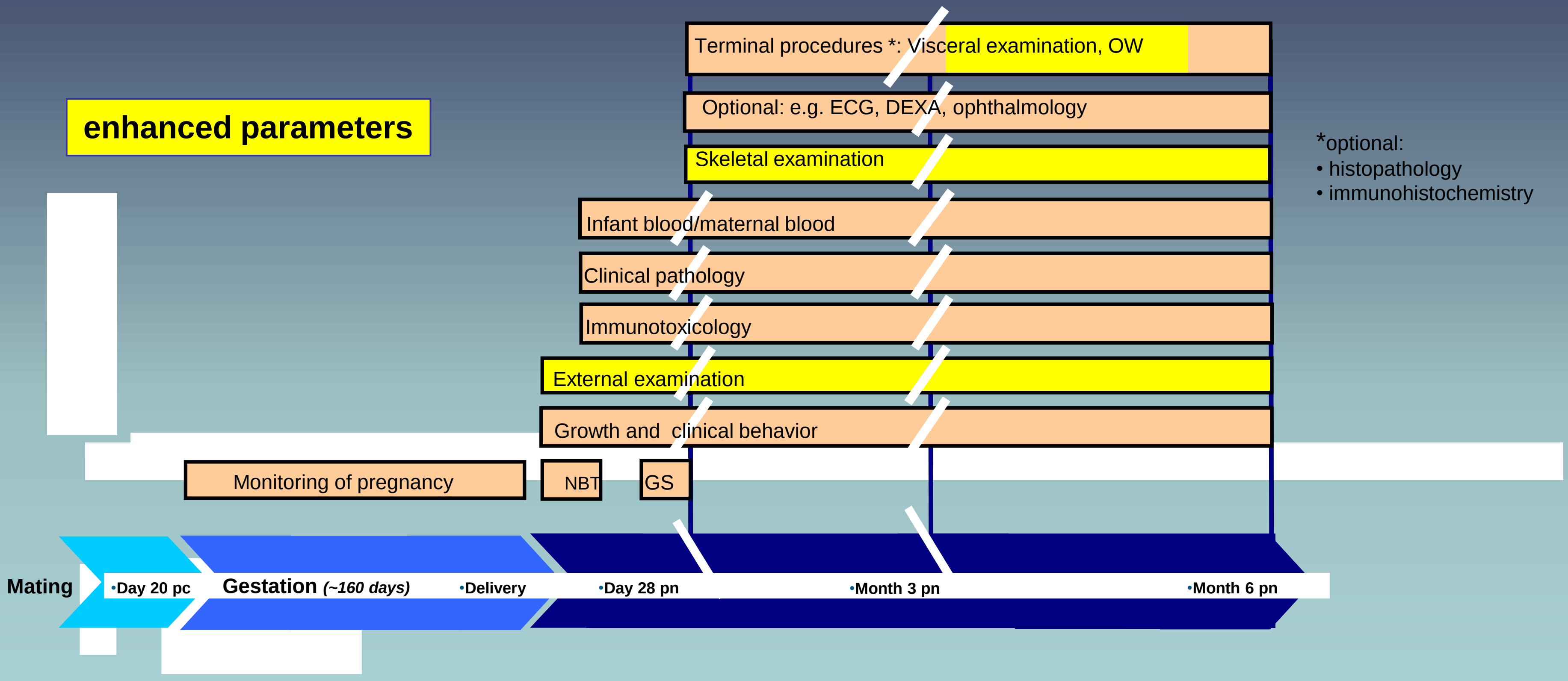
Weinbauer & Korte (2015)

Special Planning for Juvenile NHP Supply



Weinbauer & Korte (2015)

ICH S6 (R1) - Enhanced PPND Design



NBT = neurobehavioural test battery, GS = grip strength, ECG = electrocardiography, OW = organ weights, DEXA = bone density & body mass

Weinbauer et al (2011) Birth Def Res Part C 93:324-333

Juvenile Nonhuman Primate Model

Advantages

- Physical size facilitates collection of multiple biologic specimens
- Postnatal development of many organ systems well characterized
- Potentially less immunogenic than other species
- Standardized tests available
  - Neurobehavioral testing

Disadvantages

- Procurement of appropriately aged animals
- Not practical to test full span of postnatal development
- Limited reference or historical control data for some endpoints
- More expensive (vs rodent)
- Limited appropriate technical expertise/experience
- Statistical analysis

Summary

- **Juvenile toxicity studies in nonrodents can be done but present challenges**
  - Smaller group sizes
  - Limited historical control data
  - Technical and logistical issues
- **Understand comparative organ development and PK/ADME profiles**
- **Understand logistical requirements of study**