PreClinical Safety
CONSULTANTS LIMITED

AGAH Workshop October 2012
Reproductive Toxicology

Study for effects on pre- and postnatal development, including maternal function (ICH)

PCS, for all your outsourced preclinical safety needs

Outline

- Reminder: Aim of reproductive toxicology programme and timing conventions
- ICH: Study for effects on pre- and postnatal development (PPND study)
- Case study
  - Status of programme/general aspects
- Selected references

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Aim of reproductive toxicity programme

- The combination of studies selected should allow exposure of mature adults and all stages of development from conception to sexual maturity.
- To allow detection of immediate and latent effects of exposure, observations should be continued through one complete life cycle, i.e. from conception in one generation through conception in the following generation. For convenience of testing this integrated sequence can be subdivided into […] stages [A-F, see slide 9]
- Note 17 (4.1.2) Treatment of offspring
  - Consequent to derivation from existing guidelines for medicines this guideline does not fully cover exposures from weaning through puberty, nor does it deal with the possibility of reduced reproductive life span.
  - To detect adverse effects for medicinal products that may be used in infants and juveniles, special studies (case by case designs) involving direct treatment of offspring, at ages to be specified, should be considered.

Timing conventions (ICH Note 2)

- In this guideline the convention for timing of pregnancy is to refer to the day that a sperm positive vaginal smear and/or plug is observed as day 0 of pregnancy even if mating occurs overnight.
- Unless shown otherwise it is assumed that, for rats, mice and rabbits implantation occurs on day 6-7 of pregnancy, and closure of the hard palate on day 15-18 of pregnancy.
  - This reflects the period of organogenesis
  - Duration of pregnancy
    - Rat: 21 – 23 days (organogenesis: >50%)
    - Rabbit: approximately 29 – 31 days (organogenesis: >40%)
    - Human: 40 weeks, the first 12 weeks = period of organogenesis (ca. 30%)
- *NOTE: Rats generally mate overnight*

Timing conventions (ICH Note 2) (cont'd)

- Other conventions are equally acceptable but MUST be defined in reports. Also, the investigator must be consistent in different studies to assure that no gaps in treatment occur. It is an advisable precaution to provide an overlap of at least one day in the exposure period of related studies.
- The accuracy of the time of mating should be specified since this will affect the variability of fetal and neonatal parameters.
- […] particularly with regard to delays in, or prolongation of parturition, reference to a postcoital time frame may be useful.

NOTE: The timing of pregnancy depends on the timing of ovulation which is light dependent (in the rat). First fertilisation and start of pregnancy will always be within a couple of hours of ovulation (as long as the animals actually mate). Technically, the timing can be synchronised as much as possible by controlling when the animals get together, but rats will normally mate in the early morning hours, although this can be influenced by laboratory procedures.

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Study for effects on pre- and postnatal development, including maternal function (ICH)

- **PPND study**
  - Formerly known as "Segment III study"
    - Still in use – so if you hear this – it is an old-fashioned but very useful term ☺
- Aim
  - To detect adverse effects on the pregnant/lactating female and on development of the conceptus and the offspring following exposure of the female from implantation through weaning. Since manifestations of effect induced during this period may be delayed, observations should be continued through sexual maturity (i.e. stages C to F […] see slide 9).
- At least one species, preferably rats (ICH)
  - Standard approach presented today
- However, the monkey is more and more in use for specific medications, where the rat is not regarded as predictive for human
  - Biologics
  - Will also use specific designs (see ref. 5)

Stages of development (ICH)

A. Premating to conception (adult male and female reproductive functions,development and maturation of gametes, mating behavior, fertilisation).

B. Conception to implantation (adult female reproductive functions, preimplantation development**,** implantation).

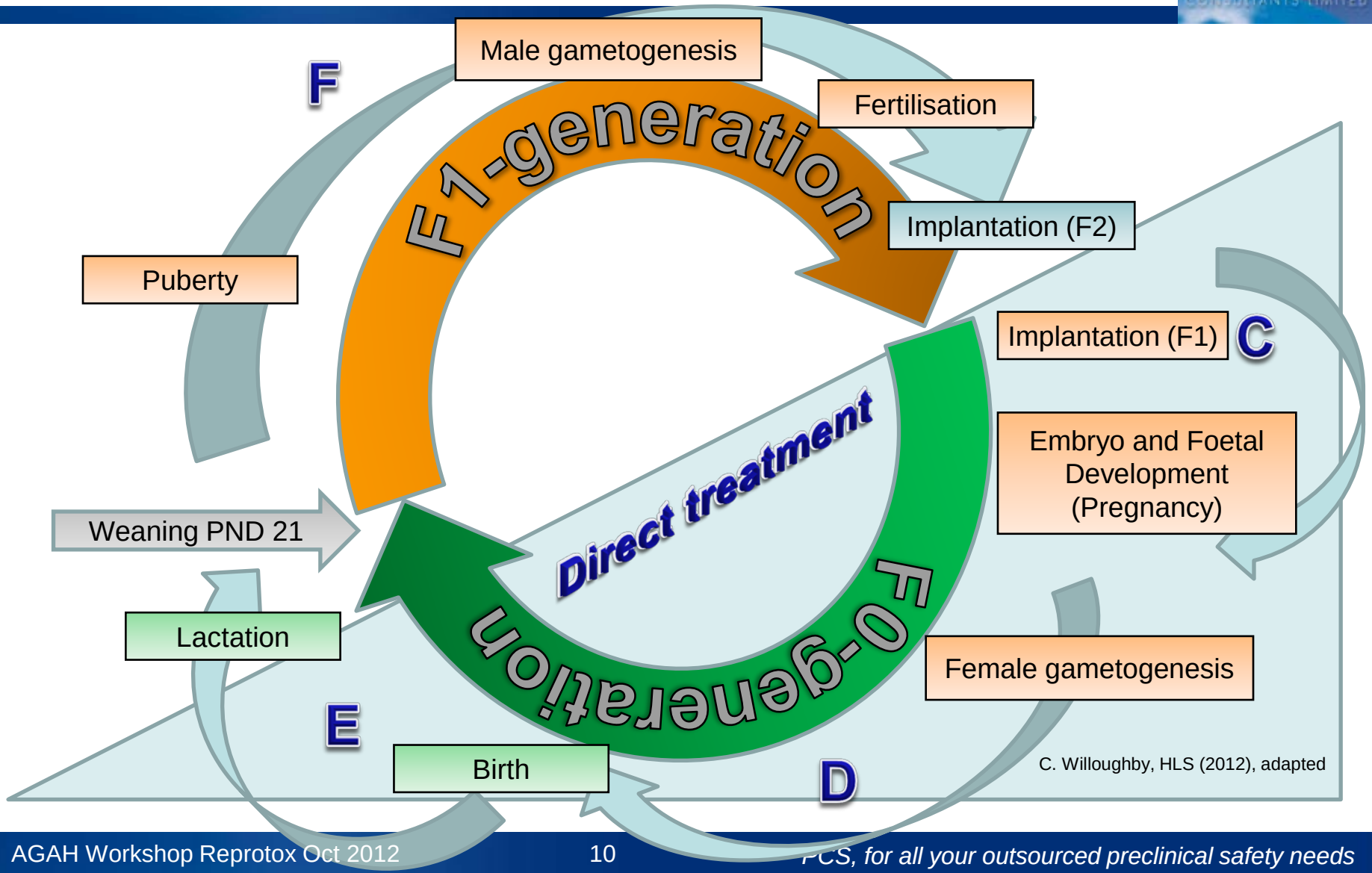
C. Implantation to closure of the hard palate (adult female reproductive functions, embryonic development, major organ formation).

D. Closure of the hard palate to the end of pregnancy (adult female reproductive functions, fetal development and growth, organ development and growth).

E. Birth to weaning (adult female reproductive functions, neonate adaptation to extrauterine life, preweaning development and growth).

F. Weaning to sexual maturity (postweaning development and growth, adaptation to independent life, attainment of full sexual function).

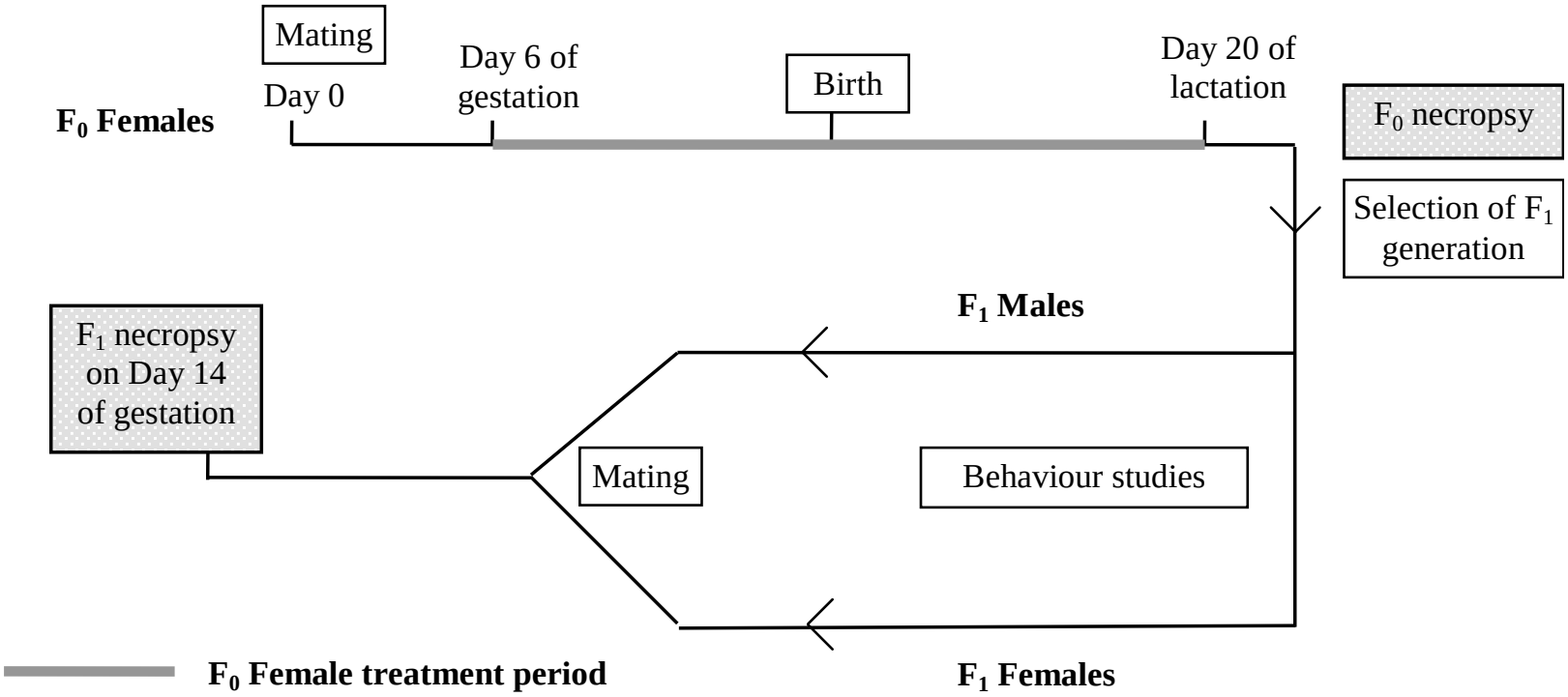
The reproductive cycle and how a PPND study works in this scheme



Adverse effects to be assessed in a PPND study

- Enhanced toxicity relative to that in non-pregnant females
  - maternal toxicity (ref. to presentation of G. Bailey)
- Pre- and postnatal death of offspring
- Altered growth and development
- Functional deficits in offspring including
  - Behaviour
  - Maturation (puberty)
  - And reproduction (F1)
- *NOTE: in addition to the points discussed above in the guidance, the PPND study will evaluate potential effects on*
  - *Gestational length*
  - *Parturition process*
  - *Maternal care for offspring*

Study design



C. Willoughby, HLS (2012)

Study design (cont'd)

- F0 males are not part of the study
  - Only used for pairing, then withdrawn, not treated
- F0 females on their designated day 0 of gestation
  - Randomly allocated to study groups
- Treatment of F0 females (dams)
  - From implantation to the end of lactation (day 21- 23 of life)
  - Stages C to E
- 16 – 20 litters/group
  - Requires higher number of mated animals
  - Pregnancy rates may be < 100%

PPND study endpoints

- In-life observations (F0 and/or F1)
  - Clinical signs and mortalities once daily
  - Body weights/food consumption
  - During mating: vaginal smears daily
  - Observations that proved of value in other toxicity studies
  - Duration of pregnancy
  - Parturition

PPND study endpoints (cont'd)

- At necropsy (for maternal animals and offspring, where applicable)
  - Necropsy (macroscopic examination) of all adults
  - Preserve organs with macroscopic findings for possible histopathological examination + corresponding organs of sufficient controls for comparison (!)
  - Implantations
  - Abnormalities
  - Live/dead offspring at birth
  - Body weight at birth

PPND study endpoints (cont'd)

- Offspring
  - Pre-weaning and post-weaning survival and growth/body weight, maturation and fertility
  - Physical development
  - Sensory functions and reflexes
  - Behaviour

Note 21 (4.1.2) Physical development, sensory functions, reflexes, and behavior

- Best indicator of physical development
  - Bodyweight development
- Achievement of pre-weaning landmarks highly correlates with body weight
  - Milk uptake (PND – post-natal day 1- 4)
  - Pinna unfolding (PND 1 of age until occurrence)
  - Coat growth (PND 5 onwards)
  - Incisor eruption (PND 10 onwards)
  - Eye opening (PND 11 of age until occurrence)
- Reflexes also dependent on physical development
  - Surface righting (PND 2 onwards)
  - Auditory startle (PND 15 or later)
  - Air righting (PND 16 onwards)
  - Response to light (pupillary light reflex) (PND 20/21)

Note 21 (4.1.2) Physical development, sensory functions, reflexes, and behavior (cont'd)

- Post-weaning landmarks (indicators of sexual maturity)
  - Vaginal opening (PND 28/29/30 onwards)
  - Cleavage of balanopreputial gland (PND 38 - 40 onwards)
- Functional tests (examples)
  - A number of different tests available, considerable differences between laboratories with respect to method; some differences with respect to timing.
  - Sensory functions e.g.
    - Auditory function (around PND 36, Preyer reflex)
    - Motor activity (e.g. open field, PND 22)
    - Neuromuscular function (Rotarod, PND 26 - 28)
    - Learning and memory (Water/Swimming/Morris Maze, PND 31 or 35 and 42)
- Some endpoints not very sensitive

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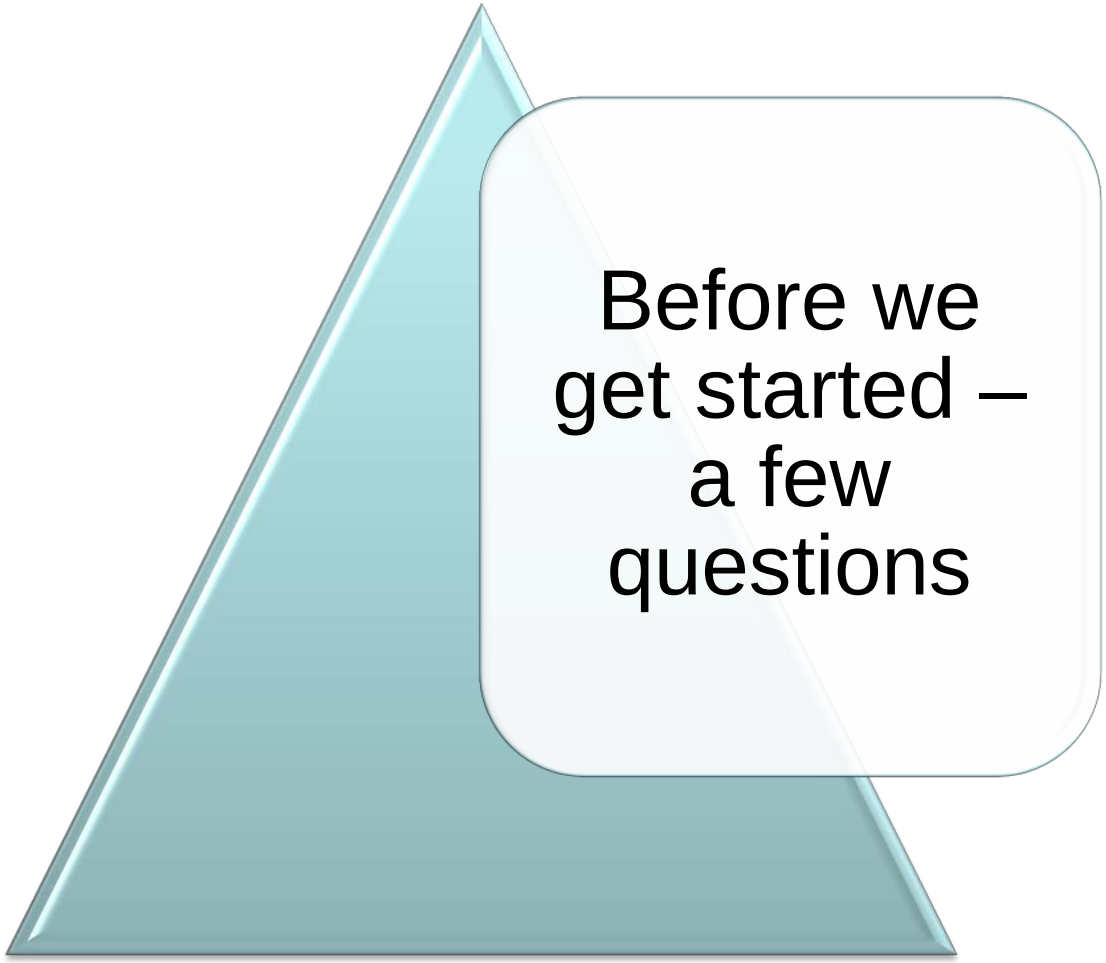
CASE STUDY



Status of your programme

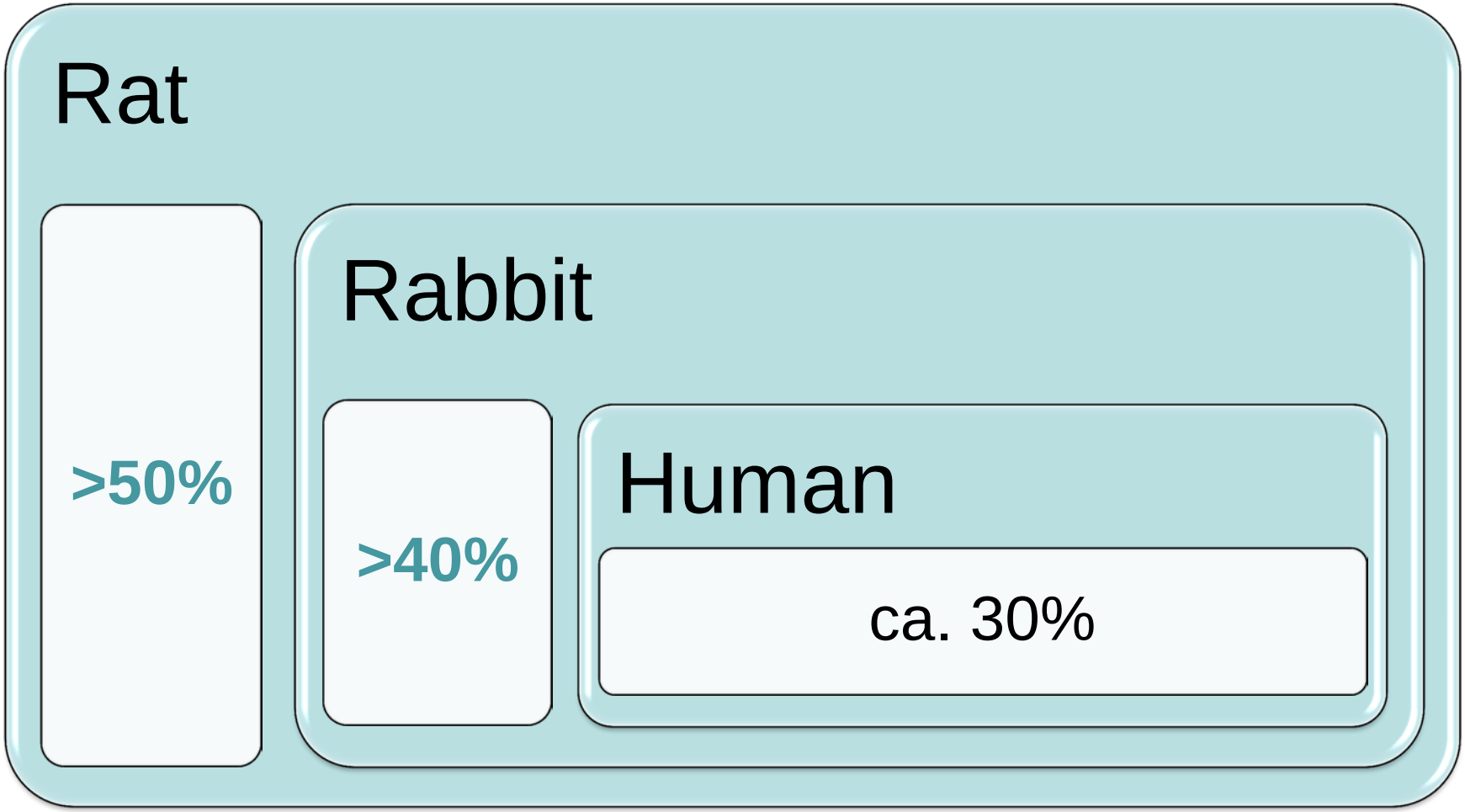
At the time when you start a PPND study, the reproductive programme will be fairly complete

All other reproductive toxicity studies will have been conducted

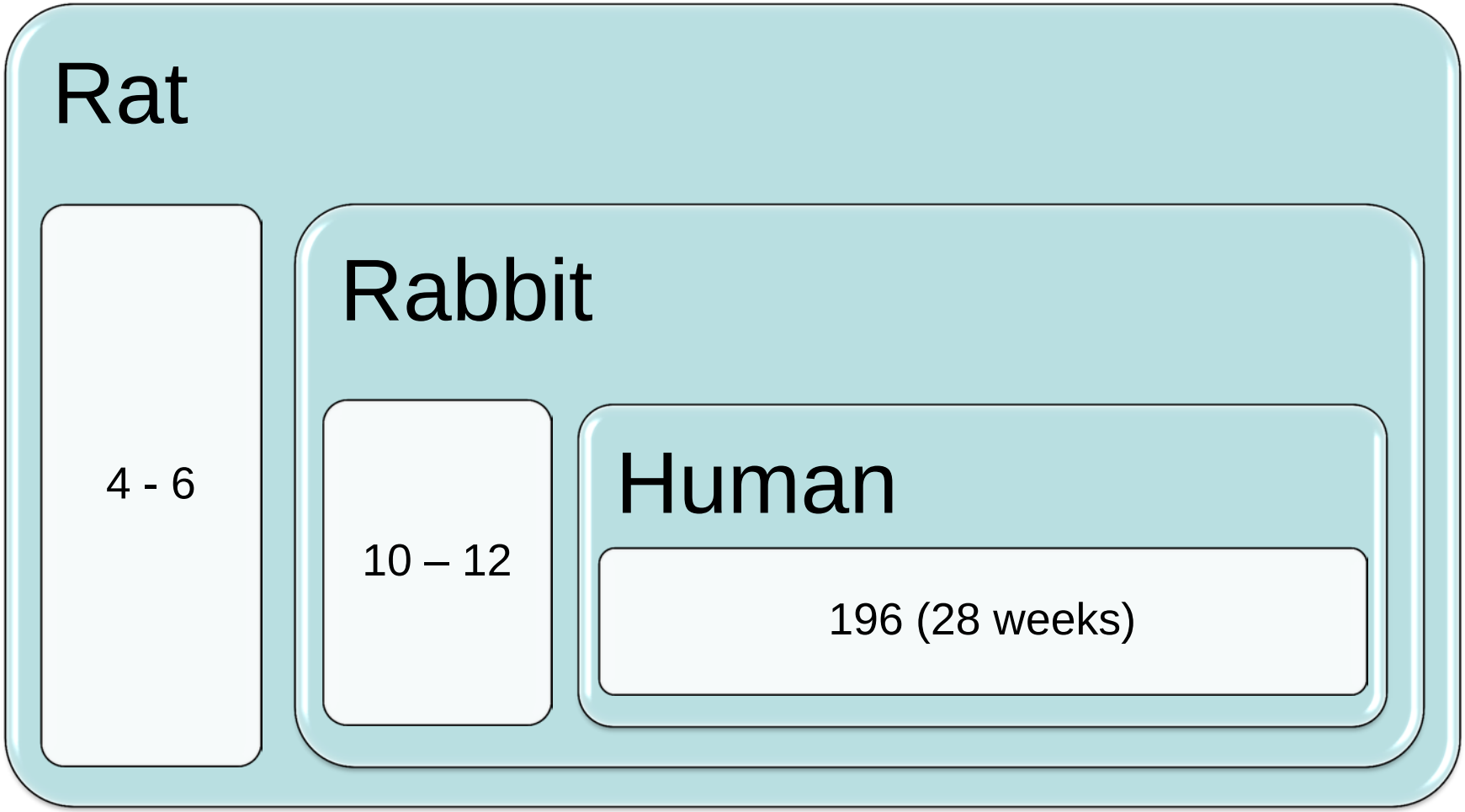
In particular, your studies for embryo-fetal development **(EFD studies)** in two species will have been finished

Reminder: Relative duration of organogenesis

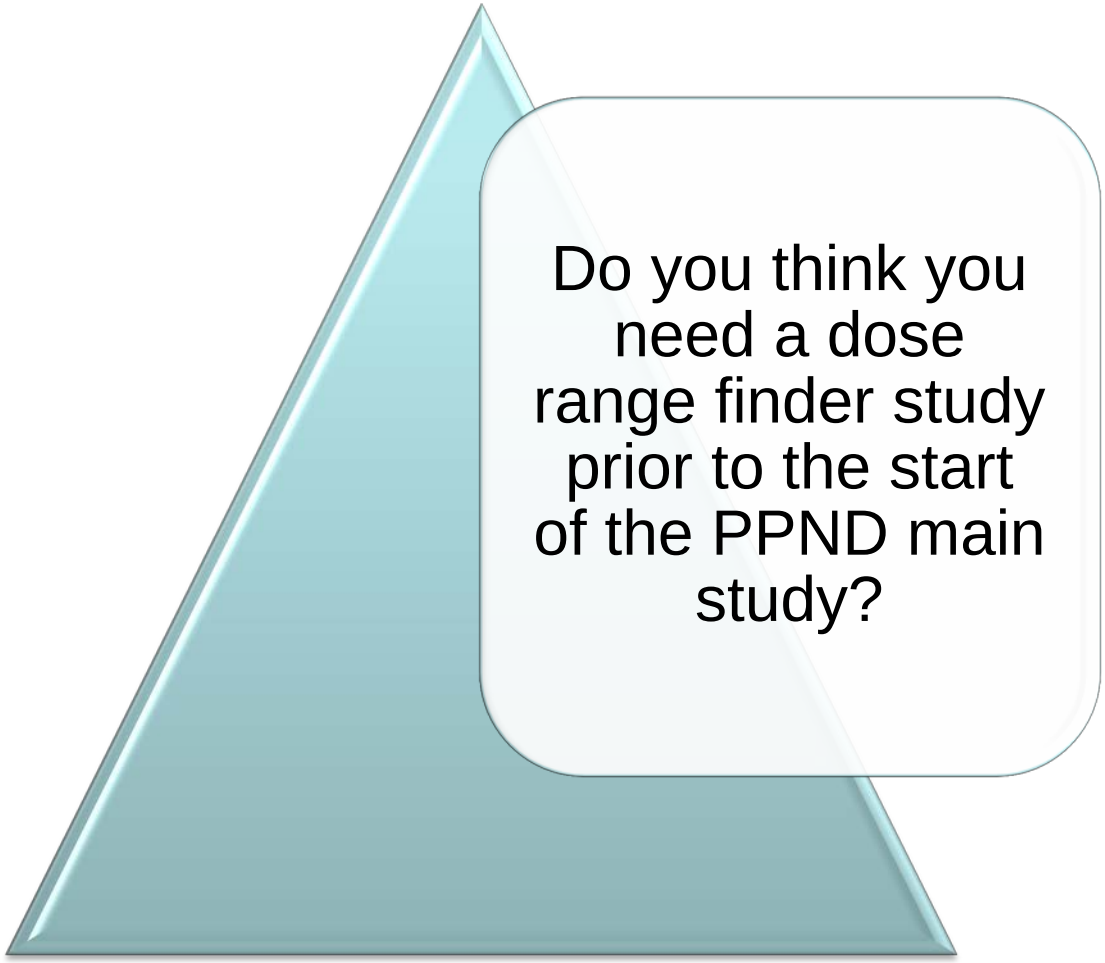
Proportion of pregnancy covered in EFD studies



Remaining days of pregnancy at the end of organogenesis [ca. days]



Rat is your model – 4 days to go before delivery



What do you want to know before you start your main study?

Change in toxicokinetic profile?

Change in sensitivity towards toxicology?

Do you think that EFD studies will give you sufficient information?

Do 4 days matter?

Maternal level?

Offspring?

Late foetal development?

Parturition?

Many thanks for your attention!

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Selected references for further reading

(1) ICH harmonised tripartite guideline: Detection of toxicity to reproduction for medicinal products & toxicity to male fertility. Parent Guideline 24 June 1993, Addendum 9 November 2000, incorporated in November 2005

(2) Gary E. Schoenwolf et. al.: Larsen' Human Embryology: Gametogenesis, Fertilization and First Week, 4th edition, (2008) pp. 15 – 50

(3) Sahoo J et al.: Behavioral and Developmental Changes in Rats with Prenatal Exposure of Mirtazapine. Sci Pharm. (2010) 78: 451–463

(4) Dommet EJ and Rostron CL: Abnormal air righting behaviour in the spontaneously hypertensive rat model of ADHD. Exp Brain Res (2011) 215: 45–52

(5) Weinbauer GF, Fuchs A, Niehaus M, Luetjens CM.: The enhanced pre- and postnatal study for nonhuman primates: update and perspectives. Birth Defects Res C Embryo Today. (2011 Dec) 93(4): 324-33

Back-up slides

ePPND Monkey study

- Rather a modified EFD study with additional post-natal endpoints
- Will not be a PPND study like in the rodent with an F1 generation followed through to implantation
  - Sexual maturity in monkey achieved at ca. 3 – 6 yrs of age
  - Pursuing F1-generation through puberty and sexual maturation into F2-generation would result in a study duration of 4 – 8 years
- Key elements of EFD investigated in new-borns/infants rather than foetus
- Post-natal development
- See reference 5 for details

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