TOPRA Annual Veterinary Symposium

Reproductive Toxicity Testing: What and Why?

A presentation by Sally Clode, Principal Reproduction Toxicologist, Sequani Limited

In this presentation we will cover

- The aims of reproduction toxicology studies
- ICH Guidelines
- The basic reproductive cycle
- How certain drugs can affect the cycle
- Study designs
- Biotechnology products
- Clinical Trials
- Labelling
- Summary
- Data presentation.

Aim of Reproduction Toxicology Studies

ICHS5 Guideline:

"The studies conducted should allow exposure of mature adults and all stages of development to sexual maturity"

ICH Guidelines

The main guideline for reproduction studies was adopted in 1993:

ICHS5

ICH HARMONISED TRIPARTITE GUIDELINE

DETECTION OF TOXICITY TO REPRODUCTION FOR MEDICINAL PRODUCTS

Recommended for Adoption at Step 4 of the ICH Process
on 24 June 1993
by the ICH Steering Committee

An addendum to S5 to cover effects on male fertility was issued in 2000.

MAINTENANCE OF THE ICH GUIDELINE ON TOXICITY TO MALE FERTILITY

An Addendum to the ICH Tripartite Guideline on

DETECTION OF TOXICITY TO REPRODUCTION FOR MEDICINAL PRODUCTS

Recommended for Adoption
at Step 4 of the ICH Process
on 29 November 1995 and
amended on 9 November 2000
by the ICH Steering Committee

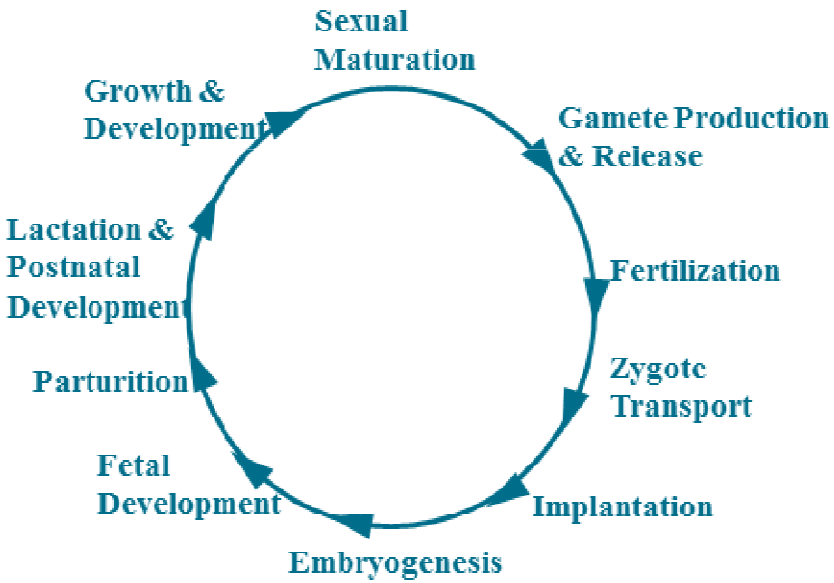
In terms of reproduction studies, the

M3(R2) 'Guidance on Non-Clinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals'

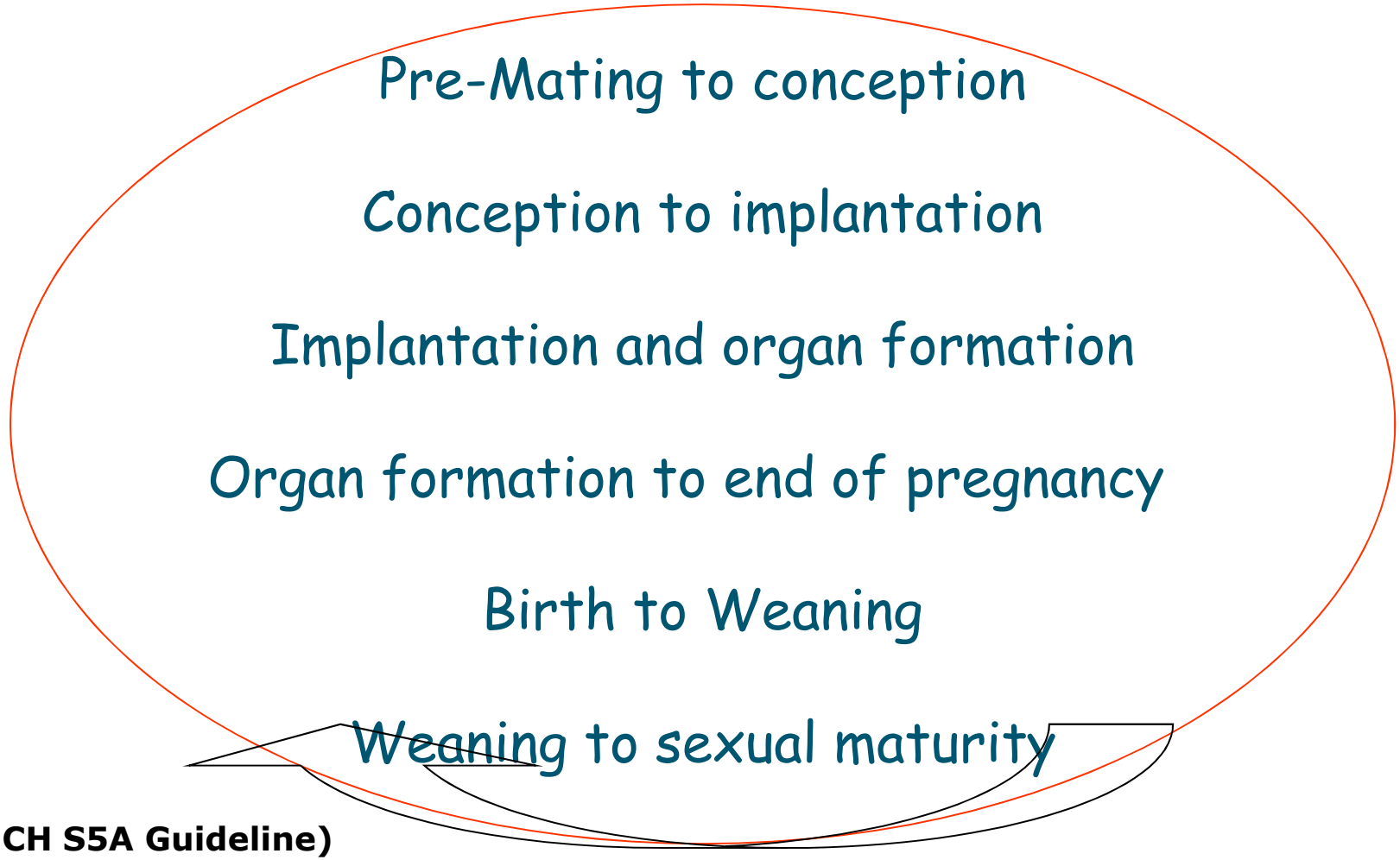
issued 2009, relaxed the requirement for non-clinical studies to support trials in women of childbearing potential.

The basic reproductive cycle

The reproductive cycle:



Within this cycle, we divide it up into an integrated sequence…….for convenience



(ICH S5A Guideline)

Drug adverse effects on different stages of the cycle:

- **Pre-mating to conception**

- Fertilisation disruption – gossypol (antimalarial)
- Abnormal sperm motility – caffeine
- Effects on libido – alcohol
- Disrupted egg release – steroid hormones

- **Implantation**

- Diethylstilbestrol (hormone)
- Anti-histamines

Drug adverse effects on different stages of the cycle (continued):

- **Foetal development**

- Abnormal foetal growth and development – alcohol
- Abnormal foetal neurobehavioural development – heroin, cocaine

- **Foetal malformations**

- Thalidomide – anti-emetic
- Gossypol – antimalarial
- DES – synthetic oestrogen
- Ergotomine – migraine
- Aspirin – acute pain

Drug adverse effects on different stages of the cycle (continued)

- **Birth**

- Premature birth (miscarriage) – ergotomine
- Delayed birth – progesterone
- Foetal difficulties – aspirin

- **Lactation** – abnormal development of the offspring due to either direct exposure during pregnancy or transfer via milk

- Alcohol
- Anti-depressants
- Methyl mercury

Drug adverse effects on different stages of the cycle (continued)

- **Sexual maturation**

- Altered sperm maturation – colchicine (spindle inhibitor)
- Lower sperm count – cyclophosphamide
- Altered ovulation – testosterone propionate

Study Designs

The ICHS5 guideline recommends:

- flexibility – study design
- avoid suffering
- minimum number of animals necessary
- make use of existing data

For most medicinal products, the ICH recommend that the '3-study design will usually be adequate for rodents' which covers all stages of development

3-Study Design

1. Fertility and early embryonic development -Rat
   - Pre-mating to implantation
2. Embryo-Foetal development – Rat and Rabbit
   - Organogenesis
3. Pre- and postnatal development – Rat
   - Implantation to weaning

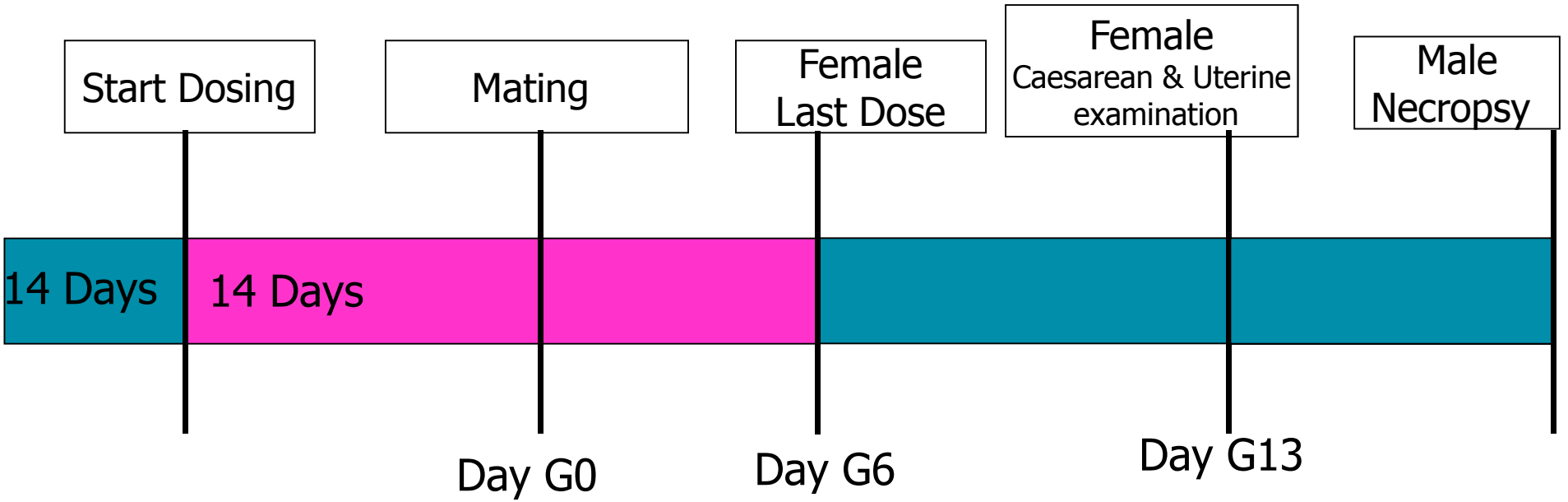
Studies may be combined

1. Fertility and Early Embryonic Development Study

- Usually conducted in rats
- May be done separately – male or female fertility assessment – or together
- Also known as Segment I study
- Multi-faceted & complex!
- Male & Female gamete production and release
- Appropriate psycho-sexual behaviour for mating
- Pre-implantation stages of the embryo
- Implantation and viability of resultant zygote

Fertility and Early Embryonic Development
Study Design

Group size = 22 Males and 22 Females



Dosing period G = Day of gestation

Endpoints in a Fertility Study

- **Males**
  - **Testicular and epididymal weights***
  - **Testicular and epididymal histopathology***
  - **Sperm assessment***
  - **Mating behaviour and pre-coital intervals**
- **Females**
  - **Oestrus cyclicity**
  - **Ovarian weight and histopathology***
  - **Number of corpora lutea, implants, live and dead embryos**
  - **Pre-coital interval**
- **And Fertility of Both!**

**** most are optional***

Embryo-Foetal Development Study

- Usually conducted in 2 species: rat and rabbit
- Also known as the Segment II study or the Teratology study
- Have to use the correct species
- at the correct time
- at the correct dose

Why 2 Species?

- Genotype influences response to exogenous agents
- Two species better than one at detecting hazard
- No species is intrinsically best at predicting for man
- Aim to have at least one pharmacologically relevant species

Species – factors for consideration

- Maternal weight
- Litter size
- Maternal basic metabolic rate
- Size and constitution of placenta
- Hormones/vitamins etc

Species - continued

RAT

For:
- **good size**
- **Highly fertile**
- **Genetic stability (background data)**

Against:
- **Low spontaneous malformation rate**
- **Low sensitivity to teratogens**
- **Sensitive to sex hormones**
- **Susceptible to NSAIDs in late pregnancy**

RABBIT

For:
- **'non-rodent'**
- **Optimal foetal size**
- **Malformation rate approx., equal to human**

Against:
- **Absence of 'pure' strains**
- **Lack of kinetic/toxicity data**
- **Susceptible to antibiotics**
- **Gastric disturbances**

Species - continued

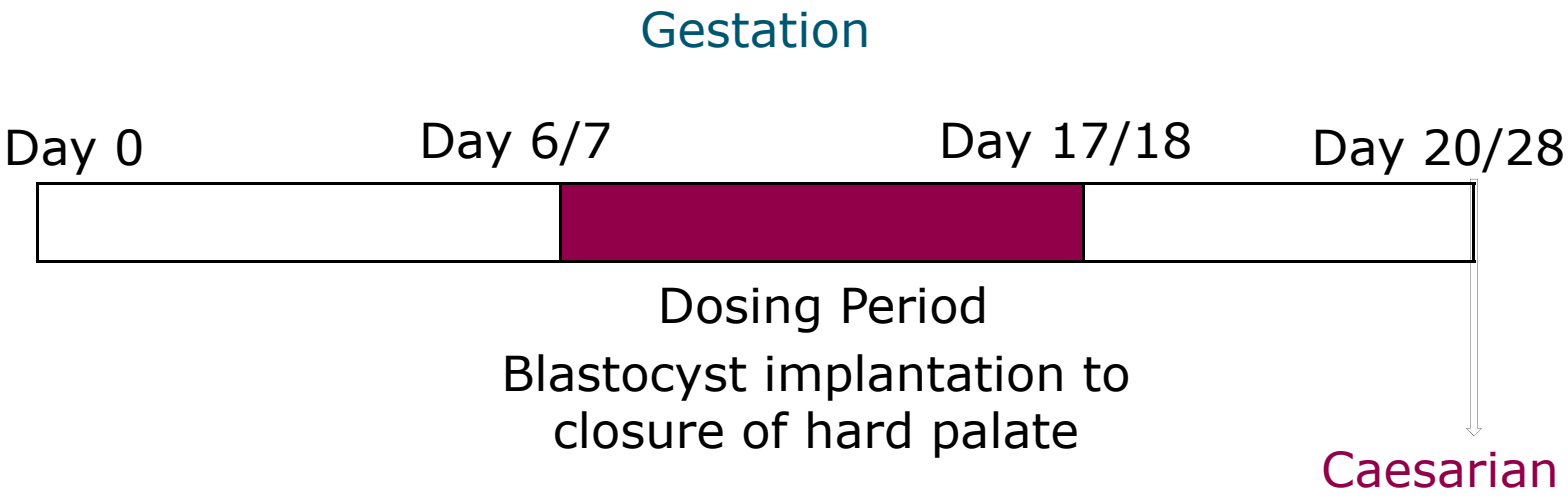
Less commonly….

Mice, mini-pigs, non-human primates, hamsters

In vitro (for special investigations) e.g. rat embryo culture and embryonic stem cells

2. Embryo-Foetal Development Study Design

Rats/Rabbits



In rats or rabbits
4 groups: 16 to 20 litters
Dams: Monitor body weights, food consumption, clinical obs etc

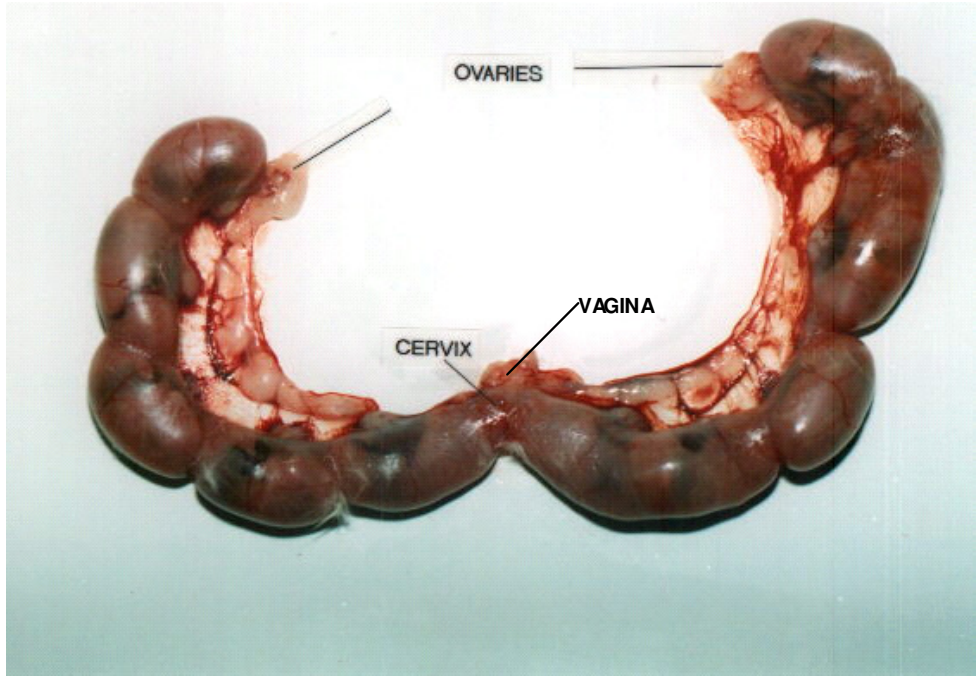
Foetal: External, soft tissue and skeletal examination

Endpoints in an embryo-foetal development study:

- **DAM**
  - Clinical observations
  - Weight gain & food consumption
  - Number of implants and foetuses
  - Post-implantation loss
- **FOETUS**
  - Foetal & placental weights
  - External abnormalities
  - Soft tissue abnormalities
  - Skeletal abnormalities
  - Death & retarded development

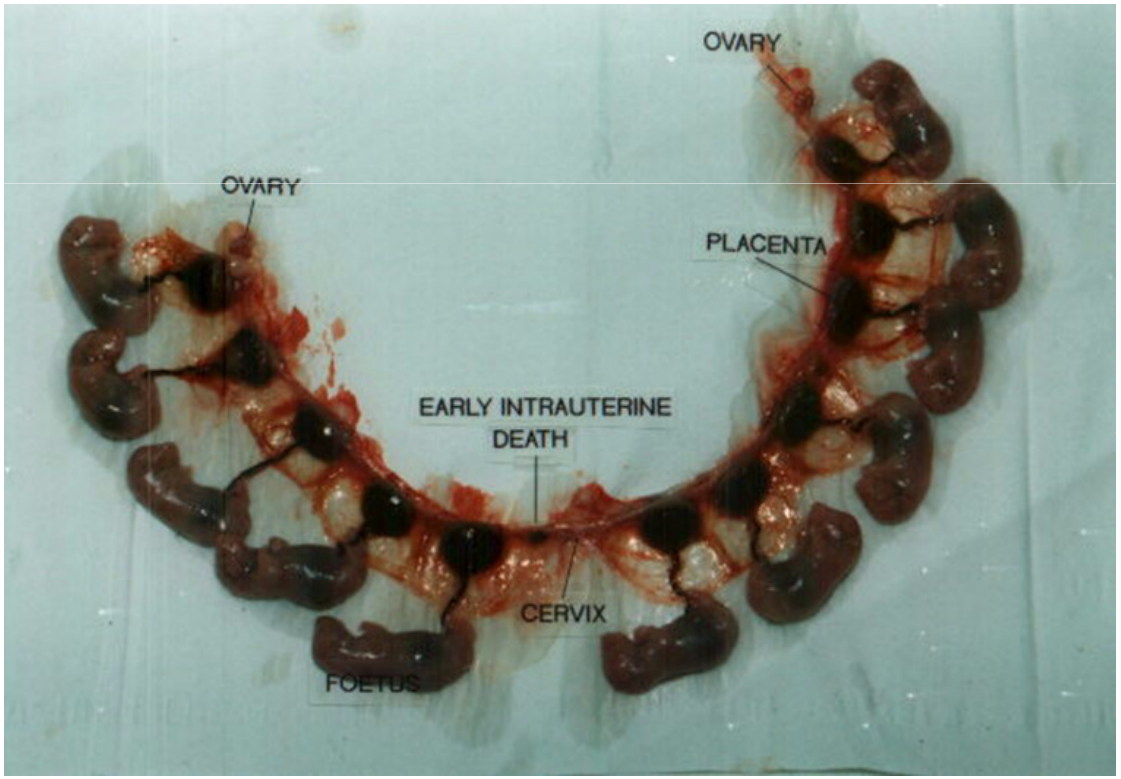
Rat Gravid Uterus

Intact uterus multiple implants



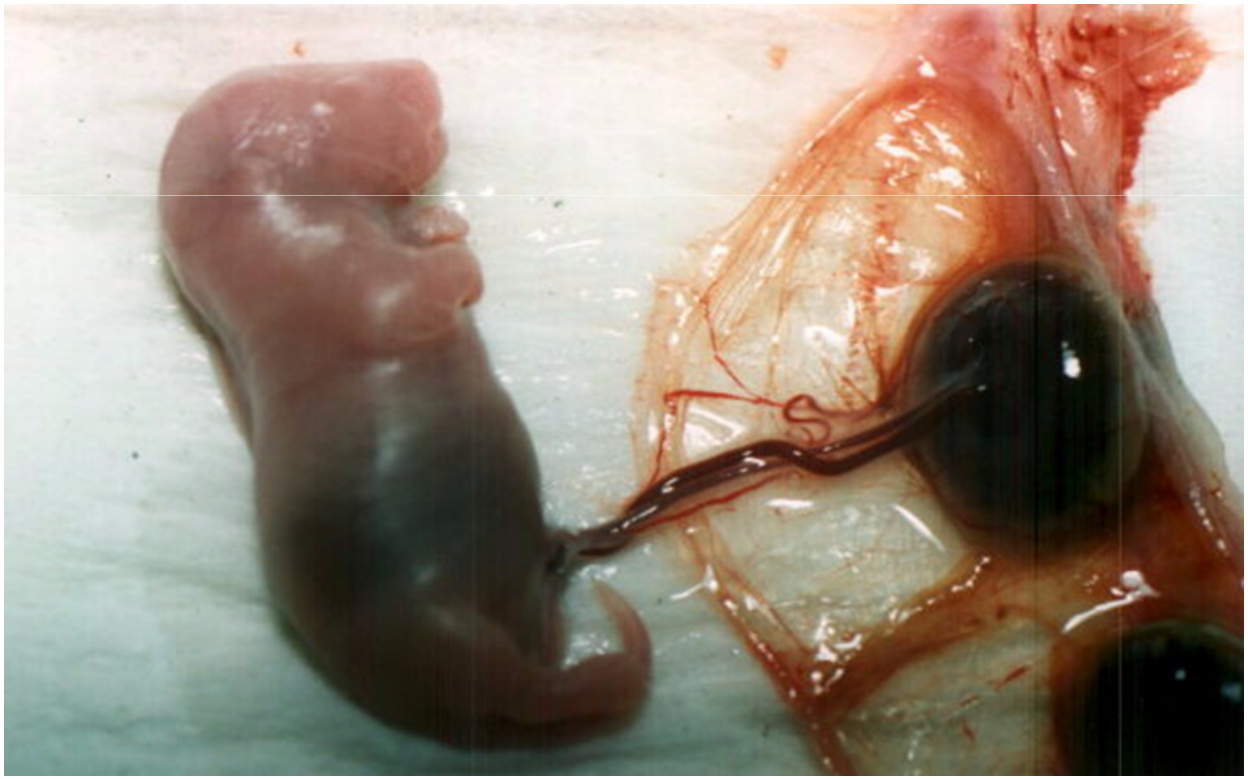
Rat opened uterine horn

Foetuses still attached to placentae



Individual Rat Foetus

Uterine horn opened – attached to placenta



Foetal Examinations

- **Soft tissue: Fresh dissection or fixed tissue**

  Bouin's fixed

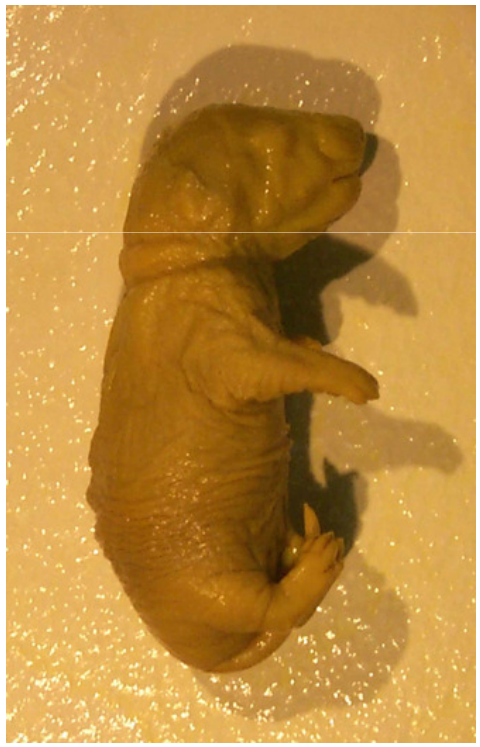
  Freehand serial sections (Wilson 1965)

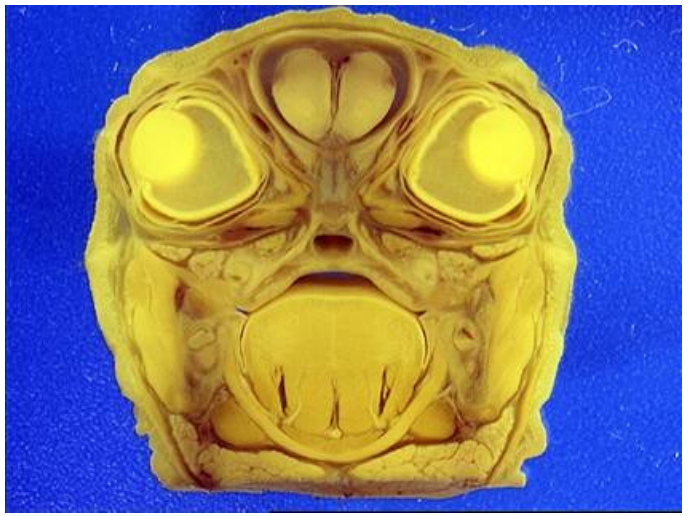
  Fixed dissection (Barrow & Taylor 1969) – more usual fixed method

- **Skeletal exam: Alizarin stained (or alizarin/alcian blue double staining)**
- **Techniques must be rapid & robust to cope with large number of foetuses**

(Up to ~20 foetuses per litter, 80 litters/study = 1000+ foetuses)

Foetal examination continued.



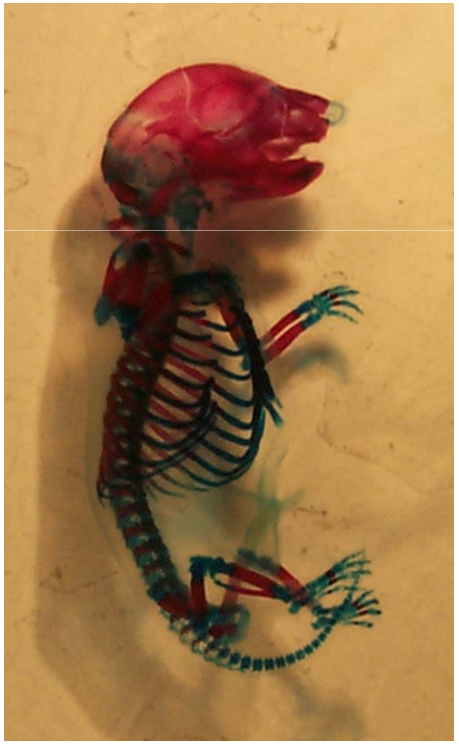


Rat Foetus – Day 20 of gestation
Bouin’s fixation to allow examination of viscera by serial sectioning

Foetal examination continued.

Rat Foetus – Day 20 of gestation
'Double Staining'

Processed and stained.
Alizarin red S (ossified) &
Alcian Blue (cartilaginous)



Interpretation of embryo-foetal study data

Triad of Effects:

- Embryo-foetal deaths, reduced foetal weight, morphologic abnormalities

Unit of statistical interest is the litter:

- Defects in a few foetuses from several litters may be more indicative of a compound effect than many affected foetuses in one litter

Compound effects can be subtle:

- e.g. delays in ossification of certain bones
  e.g. increased incidence of a normal variation

Background data are very helpful

- Tracking the occurrence of low incidence lesions in the control

  or untreated rat and rabbit aids data interpretation

Interpretation of embryo-foetal study data

Influence of maternal toxicity is controversial:

Aim is NOT to cause maternal toxicity but if foetal defects occur in dams showing toxicity, what does that mean?

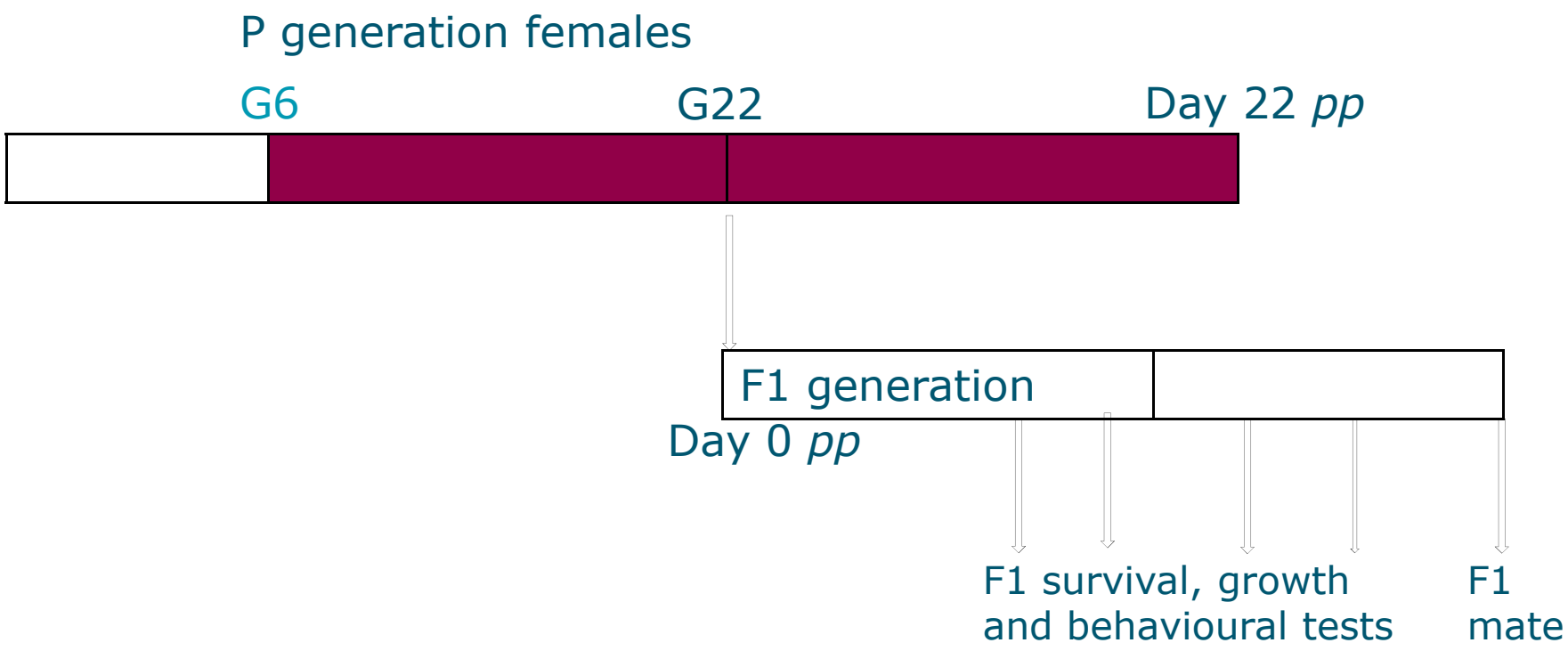
Study aim is to detect hazard, not characterise risk!

Hazard: Potential to cause harm
Risk: Likelihood to cause harm

Extrapolating from results in animals to what might happen in humans – look at all available data & assess relevance

Additional studies may be required to explain results and help determine relevance for humans – case by case basis

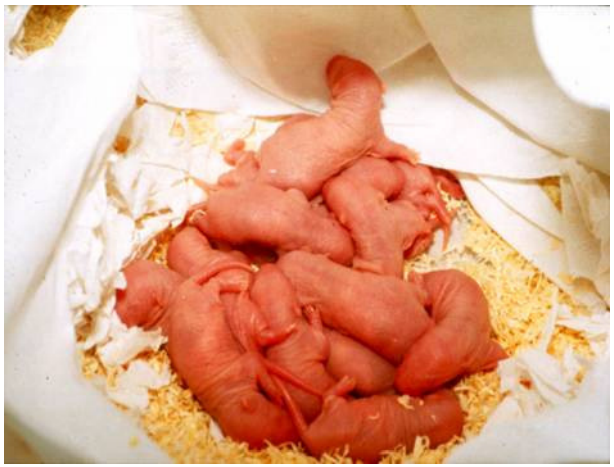
3. Pre and Postnatal Development Study Design



Rats: 4 groups – 16 to 20 litters

End-points in a Pre and Postnatal Development Study

- **Dam**
  - **Weight gain & food consumption etc**
  - **Gestation and parturition length**
  - **Changes & behaviour during lactation**
- **Pups**
  - **Survival, weight gain, sex ratio,**
  - **Physical development (pinna detachment, coat growth, locomotion, eyes open etc)**
  - **Behavioural development (motor activity, water maze learning and memory, startle responses etc)**
  - **Repro performance of F1 (to mid-gestation)**
- **Note : Doesn't deal with direct exposure of offspring from weaning – Juvenile Tox studies required for direct exposure through to maturity**



Biotechnology Derived Products

ICH HARMONISED TRIPARTITE GUIDELINE

PRECLINICAL SAFETY EVALUATION OF BIOTECHNOLOGY-DERIVED PHARMACEUTICALS

S6(R1)

Parent Guideline dated 16th July 1997

Current *Step 4* version

Addendum dated 12 June 2011 incorporated at the end of June 2011

Reproduction Studies

For products pharmacologically active only in NHPs, several study designs can be considered based on intended clinical use and expected pharmacology, Separate embryo-foetal development (EFD) and/or pre and postnatal studies (PPND), or other study designs can be appropriate, particularly if there is some concern that the mechanism of action might lead to an adverse effect on embryo-foetal development or pregnancy loss.

One well-designed study in NHPs which includes dosing from Day 20 of gestation to birth (the enhanced PPND, ePPND) can be considered, rather than separate EFD and/or PPND studies.

"Enhanced pre- and post-natal study"

Mating

Pregnancy confirmed
20 days after mating

Delivery
~160 days after mating

End of study
Infants 6 months old

Numerous pre-experimental assessments

- TK profile X2
- TK pre-dose X5
- Antibodies X3
- Immunoglobulins X3
- Clinical pathology X5
- Monitoring of pregnancy
- [Fetal growth]

Mother and Infant

- Plasma TK X4
- Milk TK X3
- Antibodies X2
- Immunoglobulins
- Clin pathology X3

Infant

- Neurobehavioural X3
- Morphological X5
- Skeletal exam X2
- Immune function X2
- ECG, opthals X1

Clinical Trials

Reproduction Studies Relative to Clinical Trials

Men

- Men can be included in Phase I (volunteers) and Phase II (patients) trials before the conduct of a male fertility study – reproductive organs are assessed in general toxicology studies.
- A male fertility study should be completed before the initiation of large scale or long duration clinical trials – Phase III

Reproduction Studies Relative to Clinical Trials

Women

- Women not of childbearing potential can be included without reproduction studies if reproductive organs are assessed in general toxicology studies.
- For women of childbearing potential there are two options:
  - Conduct appropriate reproduction studies and take appropriate precautions
  - Do no definitive reproduction studies but take precautions to prevent pregnancy by pregnancy testing, use of highly effective methods of birth control and entry to trials only after a confirmed menstrual period.

Reproduction Studies Relative to Clinical Trials

•Women of Childbearing potential

- In USA assessment of embryo-foetal development (EFD) and female fertility can be deferred to Phase III with use of adequate precautions to prevent pregnancy.
- In EU and Japan appropriate preliminary EFD studies are required in 2 species. **Definitive studies can be deferred until studies of >3 months or >150 patients are required (normally Phase III).**
  - Based on low rate of pregnancy in clinical trials
  - Adequacy of preliminary studies to detect hazard

  Female fertility studies can be deferred to Phase III
- **In all regions the pre-and postnatal study is required for marketing approval**

Clinicians should always be aware of any class history of reproductive hazard of the test material

THE LABEL

Different Approaches in USA and Europe

Purpose of Label

- **Fulfil regulatory requirements**
- **Provide guidance for physicians to make clinical decisions in the best interests of the patient**
- **Minimise liability problems – "full disclosure"**

How to Communicate Risk

- Describe potential hazard
- Interpret relevance for humans
- Indicate critical stages of pregnancy
- Give advice that takes into account the therapeutic benefit of the drug
- Update with relevant human data as available (number of pregnancies?)

Pharmaceutical Pregnancy Labelling in the USA

FDA classification (2009)

		%
A	Controlled studies show no risk	0.7
B	No evidence of risk in human	19
C	Risk cannot be ruled out	66
D	Positive evidence of risk	7
X	Contraindicated with little benefit	7

Too many in an unhelpful category !

Background incidence of defects in human approx 3%

Often difficult to get enough data to tell if real human risk or not

EU Pregnancy Label

A text based approach without defined categories

4.6 Pregnancy and Lactation

There are no adequate and well controlled studies with ‘Cure-ital’ in pregnant women. Animal studies have shown no teratogenic effects or impaired growth.

Although no data exist in humans, excretion of ‘Cure-ital’ in breast milk has been seen in animals. Caution should be exercised when considering the administration of ‘Cure-ital’ to a nursing woman.

Guideline on Summary of Product Characteristics: (Pharmaceutical Committee, December 1999)

Rules Governing Medicinal Products in the European Union, Volume 2A and 2B: Notice to Applicants

http://pharmacos.eudra.org/F2/pharmacos/docs/Doc2000/SPCGuidRev0-Dec99.pdf

SUMMARY

- **Harmonised guidelines (EU, US and Japan) exist for testing new medicinal products**
- **The entire reproductive cycle is covered by these tests; no gaps; performed during different phases of drug development**
- **Support of Clinical Trials – What and When?**
- **Risk assessment and Product Labels**

References:

INTERNATIONAL CONFERENCE ON HARMONIZATION (ICH)

- **http://www.eudra.org/humandocs/humans/ich.htm**

- **S5a – Note for Guidance on Reproductive Toxicology: Detection of Toxicity to Reproduction for Medicinal Products (CPMP adopted Sept 1993)**
- **S5b – Note for Guidance on Reproductive Toxicology: Toxicity on Male Fertility (CPMP adopted Dec, 1995)**
- **S5b Guideline on Detection of Toxicity to Reproduction for Medicinal Products; Addendum on Toxicity to Male Fertility (Nov. 2000)**
- **M3 – Note for Guidance on Non-Clinical Safety Studies for the Conduct of Human Clinical Trials for Pharmaceuticals (R2, June 2009)**

QUESTIONS?

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