How Toxicity Is Assessed ?

In vitro/animal studies

- Systemic toxicity studies (such as clinical signs and symptoms, clinical pathology, histopathology)
- Special functional tests (e.g., reproductive performance, immune system function, neurological tests)

Human studies

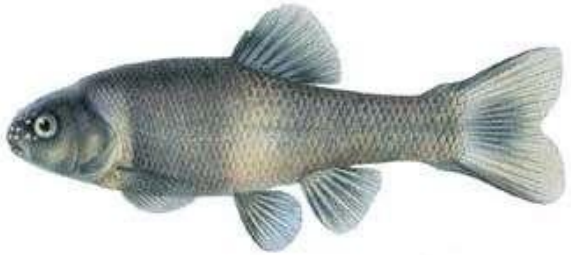
- Epidemiological studies
- Human clinical studies
- Case reports

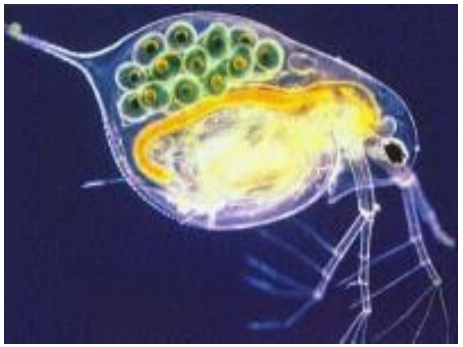
Toxicity can be

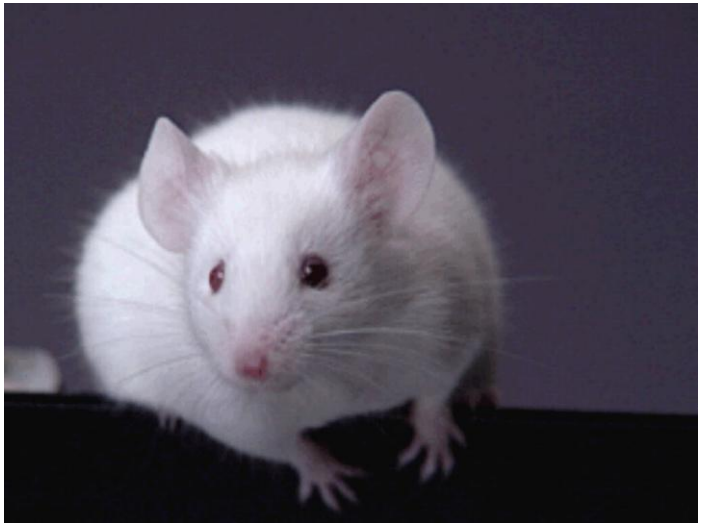
- Acute Toxicity: a toxic response ,often immediate, induced by single exposure.
- The acute toxicity of a substance is defined by its LD_{50} / lethal dose that will kill 50% of a group of exposed animals
- Chronic Toxicity: a toxic effect that requires some time to develop.
- Testing for chronic toxicity involve continuous feeding of the test substance to animals for long time (50% of animal life)

Acute Toxicity Test Organisms

- Use of test species based on
  - Lab hardiness
  - Common
  - Known life cycle
  - Cheap
  - Short-lived



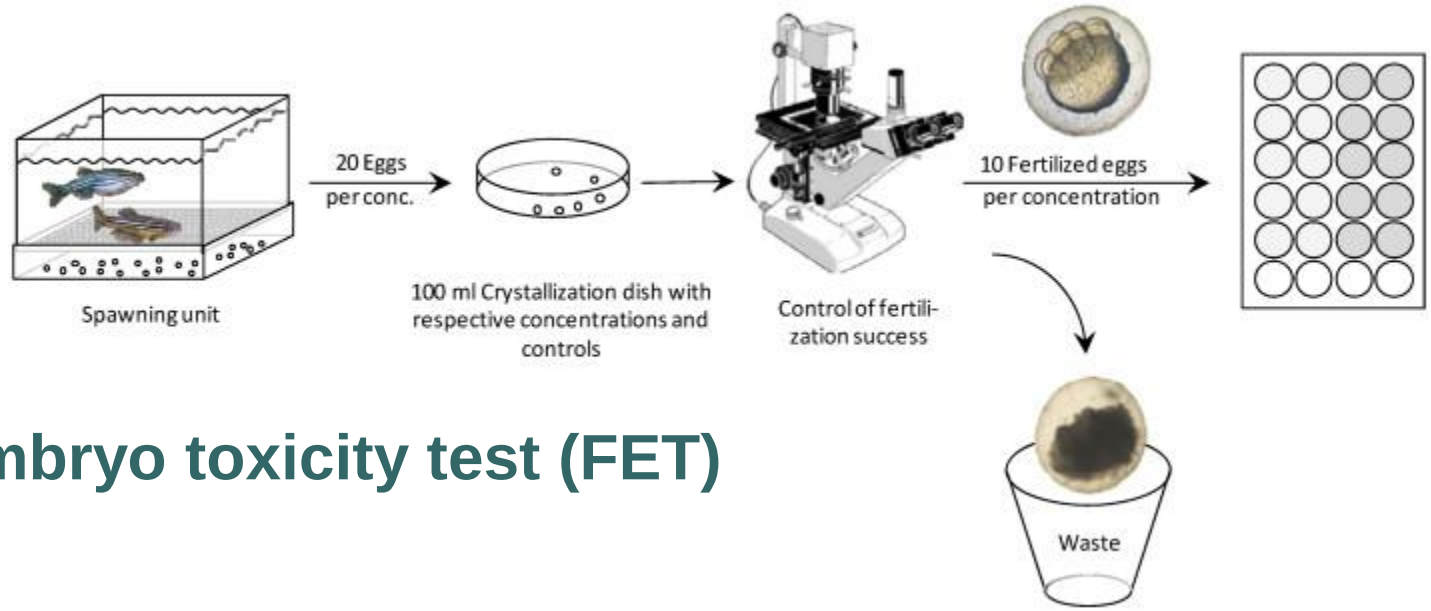




Acute toxicity testing

- Time = 2 days (invertebrates) to 4 days (fish and mice)

- Endpoints are
  - Death
  - LD50/EC50



Fish embryo toxicity test (FET)

Chronic toxicity testing

- Time = 7days to 18 months (50 % of life span)
- Endpoints are
  - growth
  - Reproduction
    - brood size (Ceriodaphnia dubia can have 2-3 broods in seven days)
    - Reproductive success
    - Teratogenicity studies (birth defects)
  - To define safety factor (NOAEL/LOAEL)

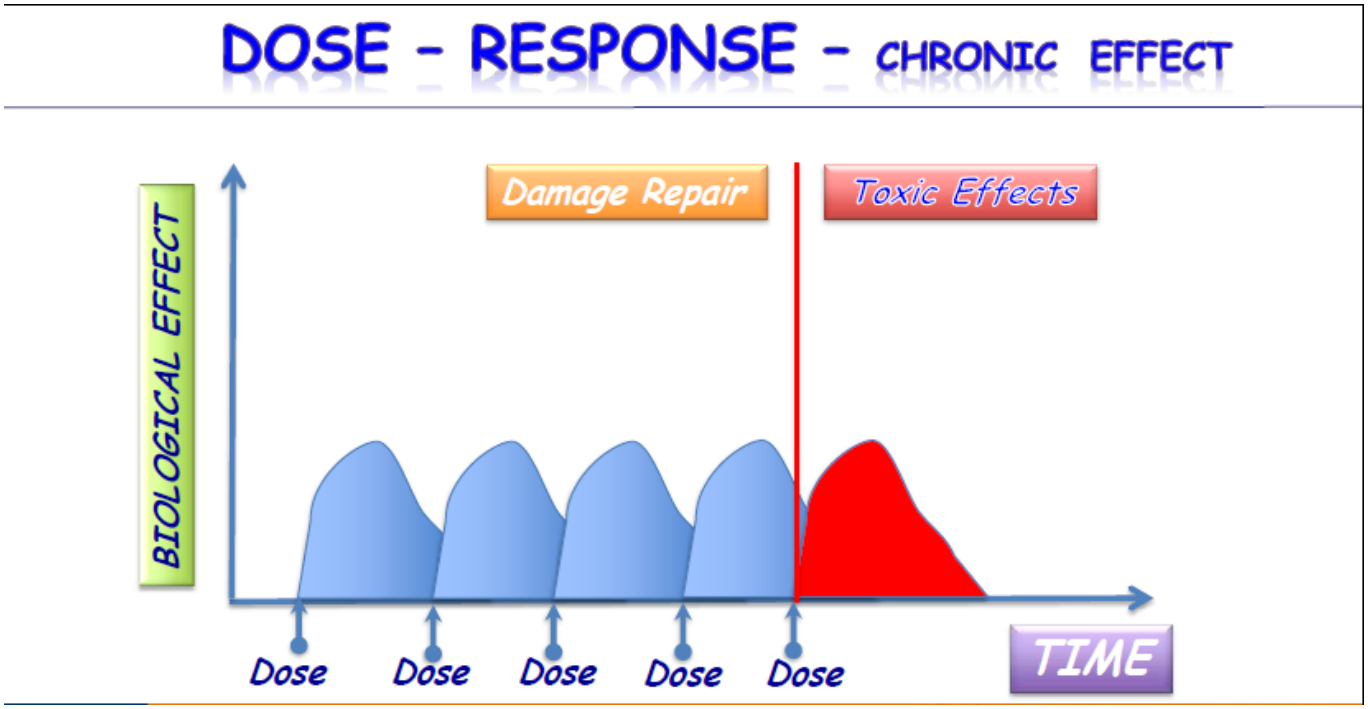
Sub chronic testing

- Repeated dose for up to 6 months
- 10 % life span
- 2 spp, rodent and non-rodent
- reflect cumulative effect, latent period and reversibility
- non-lethal parameter
- target organ arranged
- Data for chronic study

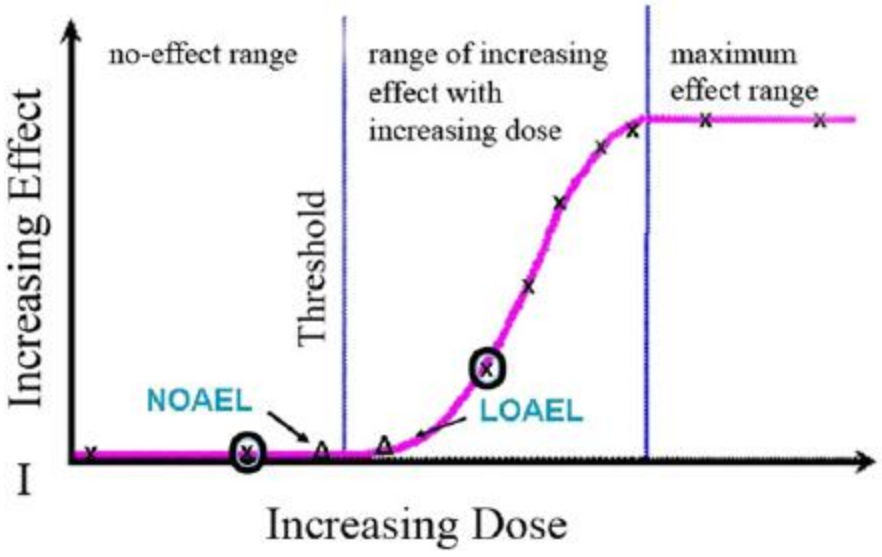
Chronic Toxicity

Studies used to determine the safe Level of Exposure

- Determine NOEL or LOAEL from dose-response curves obtained from toxicology studies
- Determine the uncertainty factors to extrapolate the results from animal studies to humans.



The dose-response curve



x Experimental doses

Δ True NOAEL and LOAEL

○ Estimated NOAEL and LOAEL

LD_{50} = 50% of species exposed to dose die (Oral route)
measured in mg/Kg

LC_{50} = 50% of species exposed to concentration die (Inhalation route)
measured in ppm or mg/m3

NOAEL – Highest dose at which there is No Observed Adverse Effect Level. Some dose response curves may not have a threshold, starting at zero.

Lowest observed adverse effect level (LOAEL)

Animal use in chronic toxicological studies

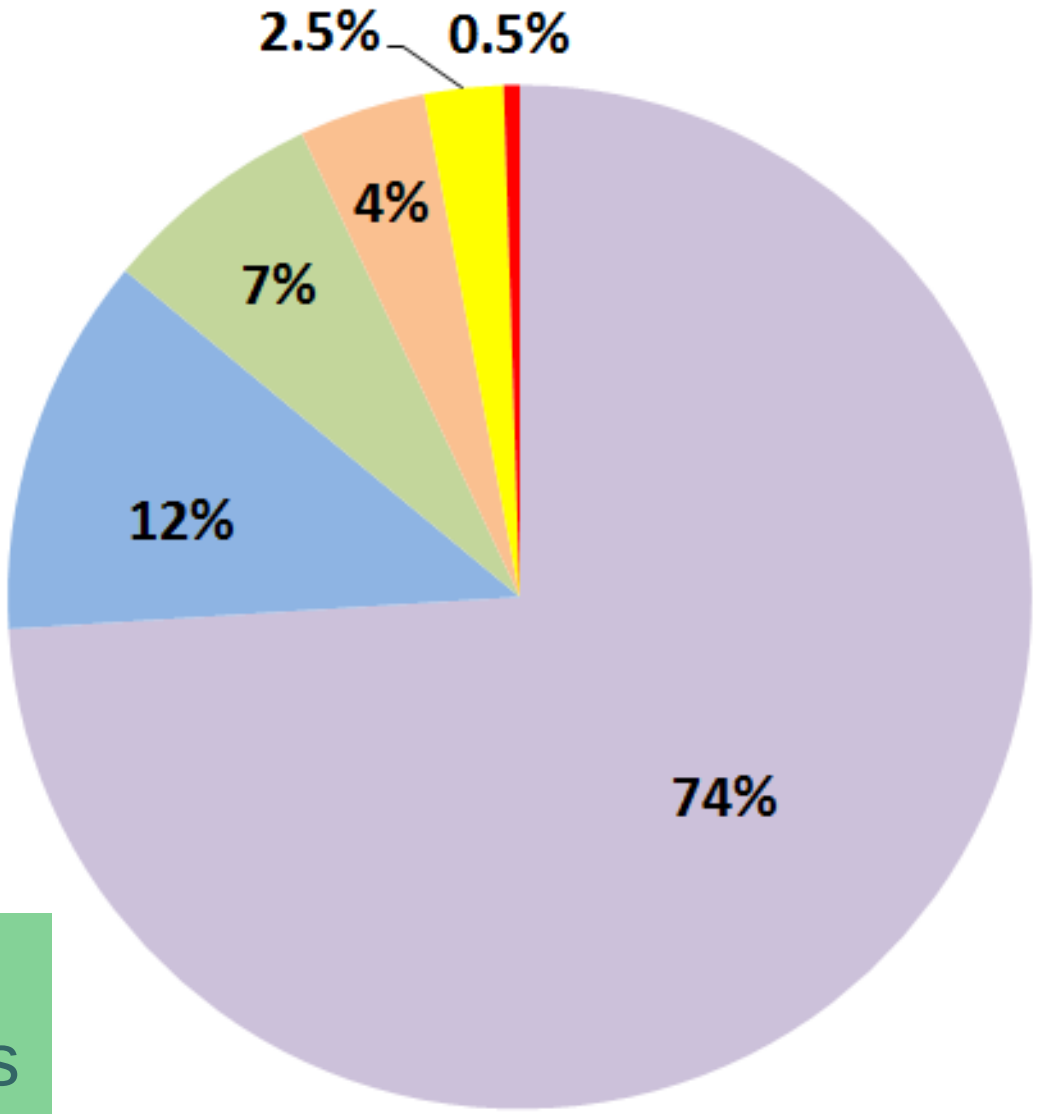
Advantages and Limitations of Using Animal Toxicity Data

Advantages	Limitations
Enable proactive regulation and behavior	Variability in results
Toxic effects assumed to be similar in humans and animals	Results in animals sometimes different from humans
Allow for control of exposure and negative and vehicle controls	Anatomical differences between humans and animals
Allow for time course	Some serious chronic effects missed by standard toxicity studies
Control/knowledge of genetically associated responses	Traditionally only study exposure to individual chemicals
Allow for invasive endpoints such as necropsy	
Less costly and timely than human exposure or epidemiology studies	

Design Aspects of Animal Toxicology Studies

	To Support Hazard Identification	To Support Dose-Response Assessment
Test material purity	Analyze and report	
Vehicle (e.g., water, corn oil, filtered air)	Consider including non-vehicle control group	Analyze and report on stability of test compound in vehicle
Animal model	• Consider conducting studies in both sexes and > 1 species • Identify susceptible lifestage(s) by conducting studies in animals of various ages • Some specific animal models can be used to identify sensitive populations	
Exposure Levels	Dose range should include a low-effect level (LOEL) for ≥ 1 health effect	• Use ≥ 3 chemical doses in addition to controls • Logarithmic spacing • Dose range should include a no-effect level (NOEL)
Statistics	Sufficient statistical power	Clearly describe statistical methods

Which Percentage Applies To Which Animals?



Fish

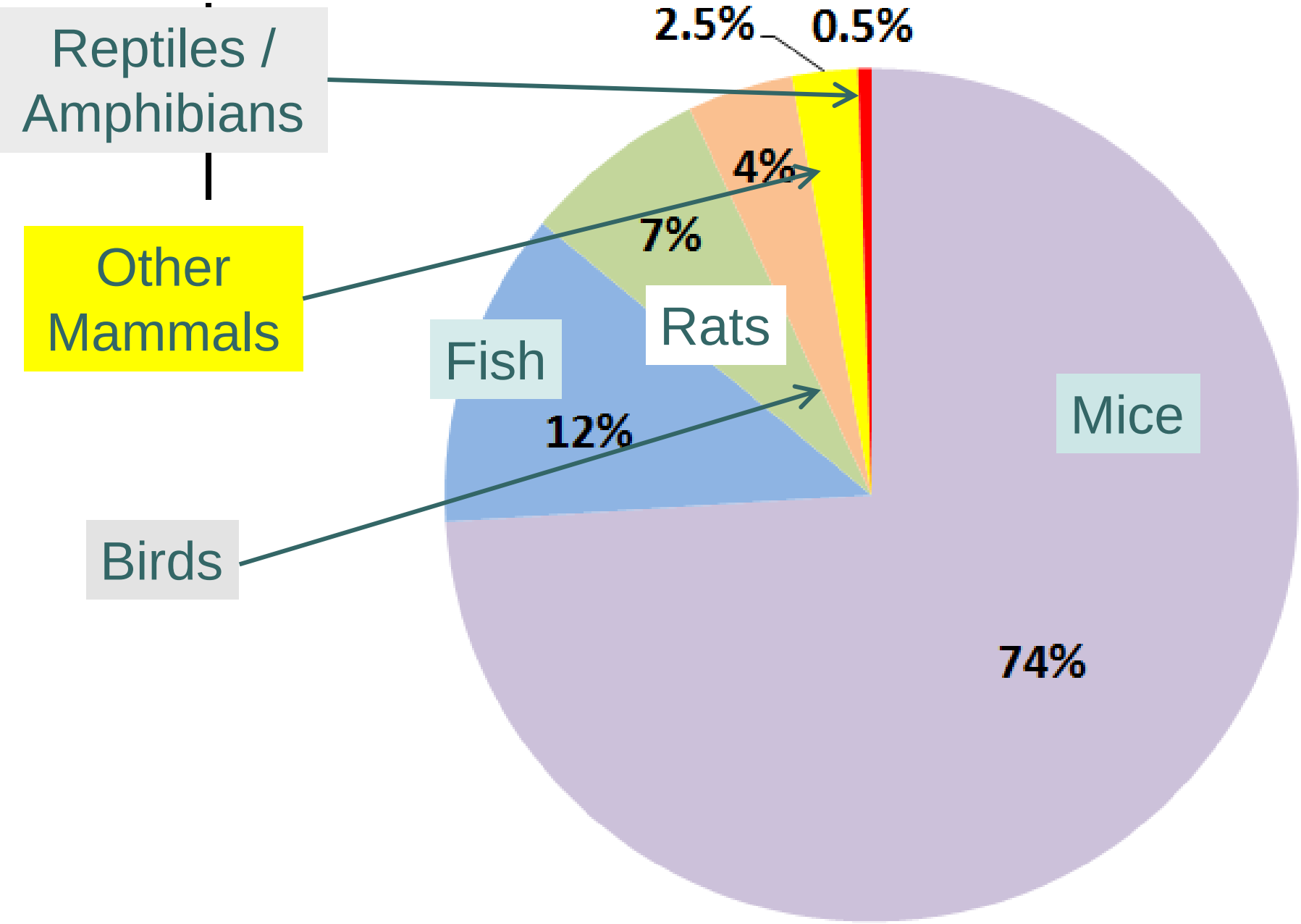
Birds

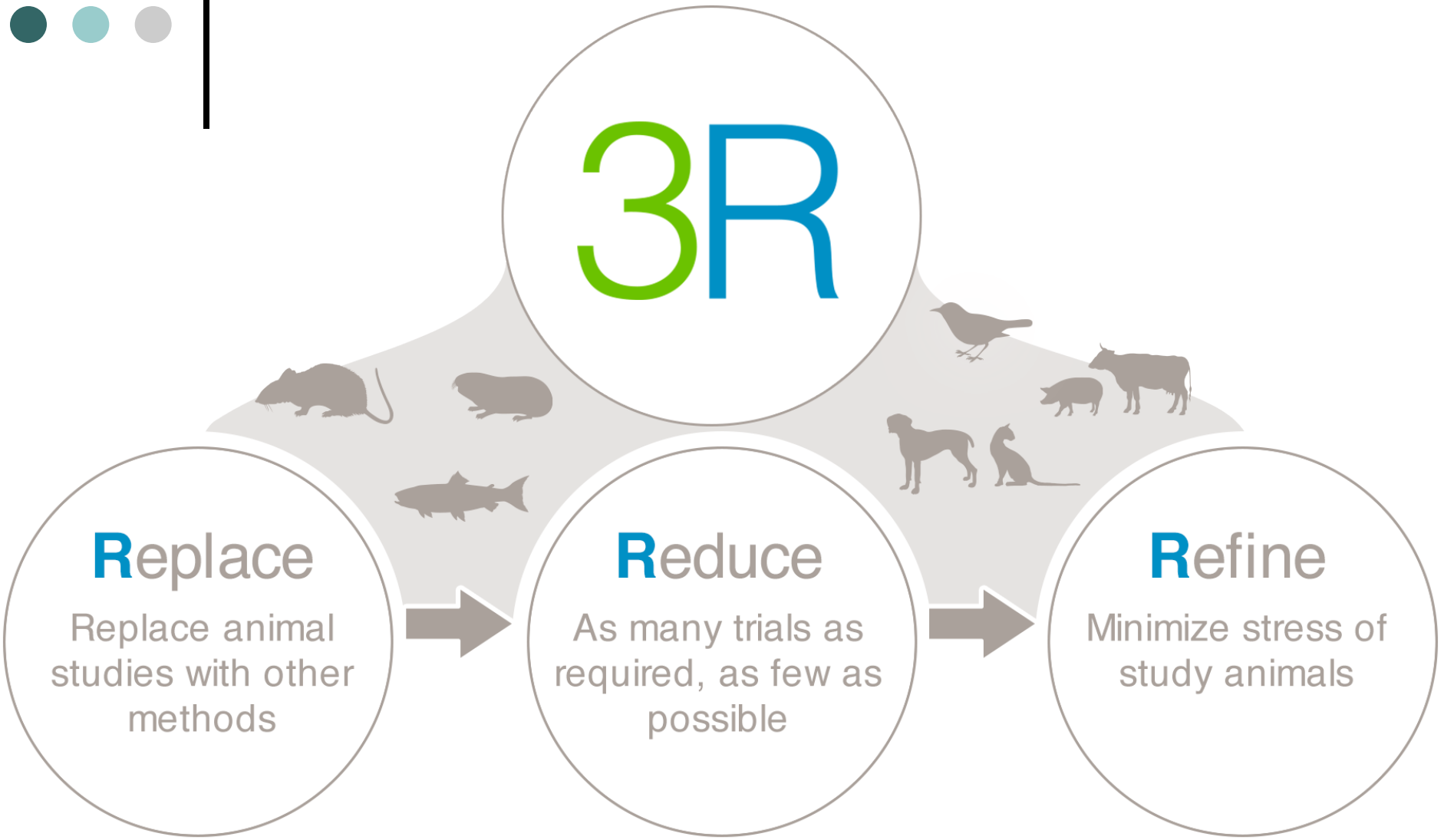
Rats

Mice

Reptiles / Amphibians

Other Mammals


Reptiles / Amphibians
Other Mammals
Fish
Birds
Rats
Mice
2.5%
0.5%
4%
7%
12%
74%


3R
Replace
Replace animal studies with other methods
Reduce
As many trials as required, as few as possible
Refine
Minimize stress of study animals

The 3Rs

The 3Rs are principles of good science designed by scientists to improve animal welfare and scientific accuracy.

Refinement – Finding ways of making animals' lives better in labs, this can include toys for animals or better training for technicians

Reduction – Using as few animals as possible to get good results

Replacement – Using non-animal alternatives wherever they exist

Reproductive toxicology tests

Endpoints are

- Reproductive efficiency
- Fertility profile (male and female)
- Teratogenetic effects

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

Linee guida internazionali (Conferenza internazionale di armonizzazione: ICH) per lo studio della tossicologia del sistema riproduttivo

Medicamento	Composto chimico
Segmento 1 (fertilità, embriotossicità) **Segmento 2** (teratogenicità) **Segmento 3** (studio peri- e postnatale)	**Teratogenicità** **Studio di una generazione** **Studio di due generazioni**

Segmento IV studi multigenerazionali

The ICHS5 guideline recommends

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

→ **The 3 Rs**

1. Fertility and early embryonic development study (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

Fertility and early embryonic development study (Segment I)

Usually conducted in rats

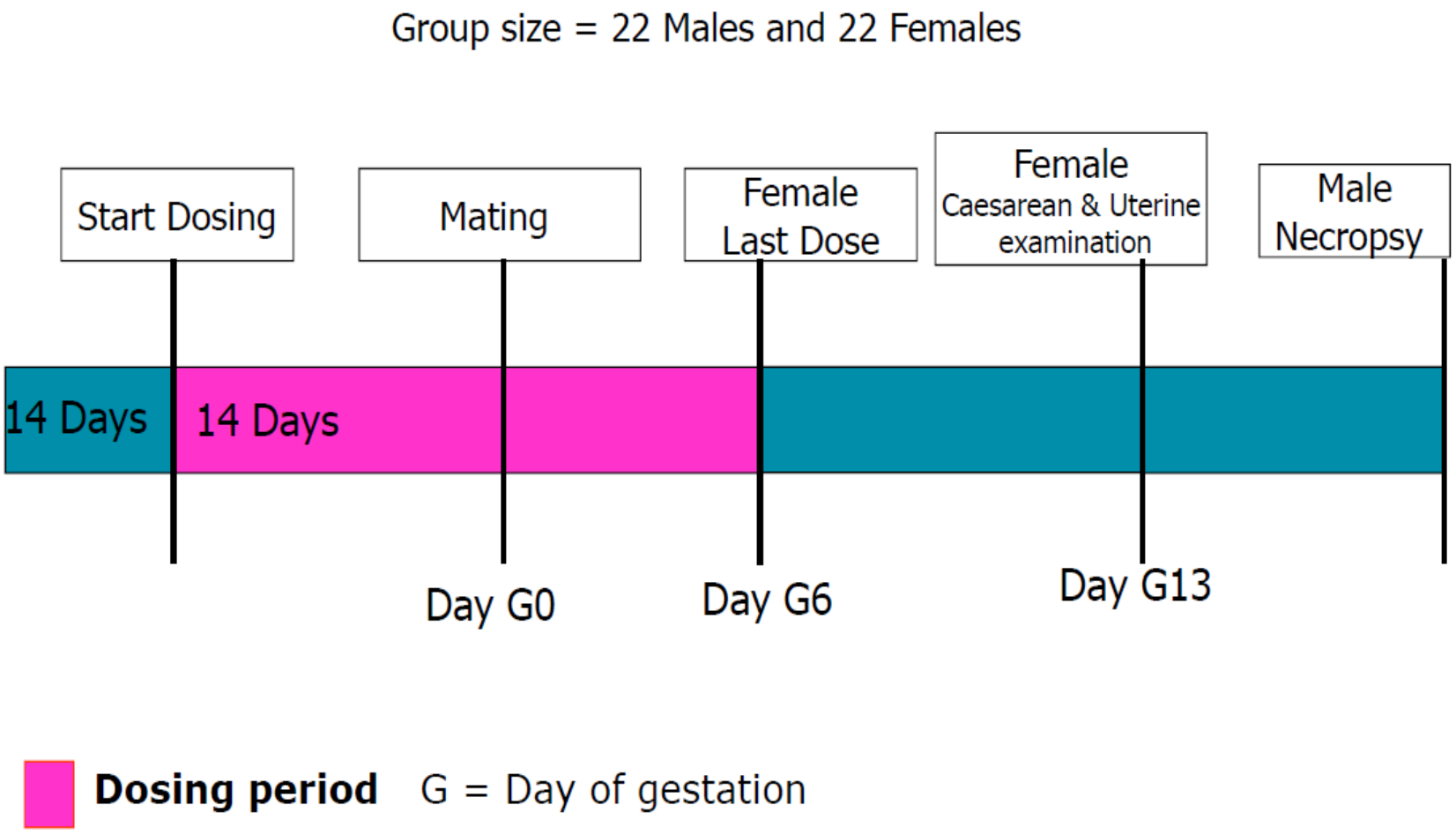
- May be done separately – male or female fertility assessment – or together
- Male & Female gamete production and release
- Appropriate psycho-sexual behaviour for mating
- Usually uses 20rats per group

– Young males treated for 60-80 days (spermatogenesisperiod)

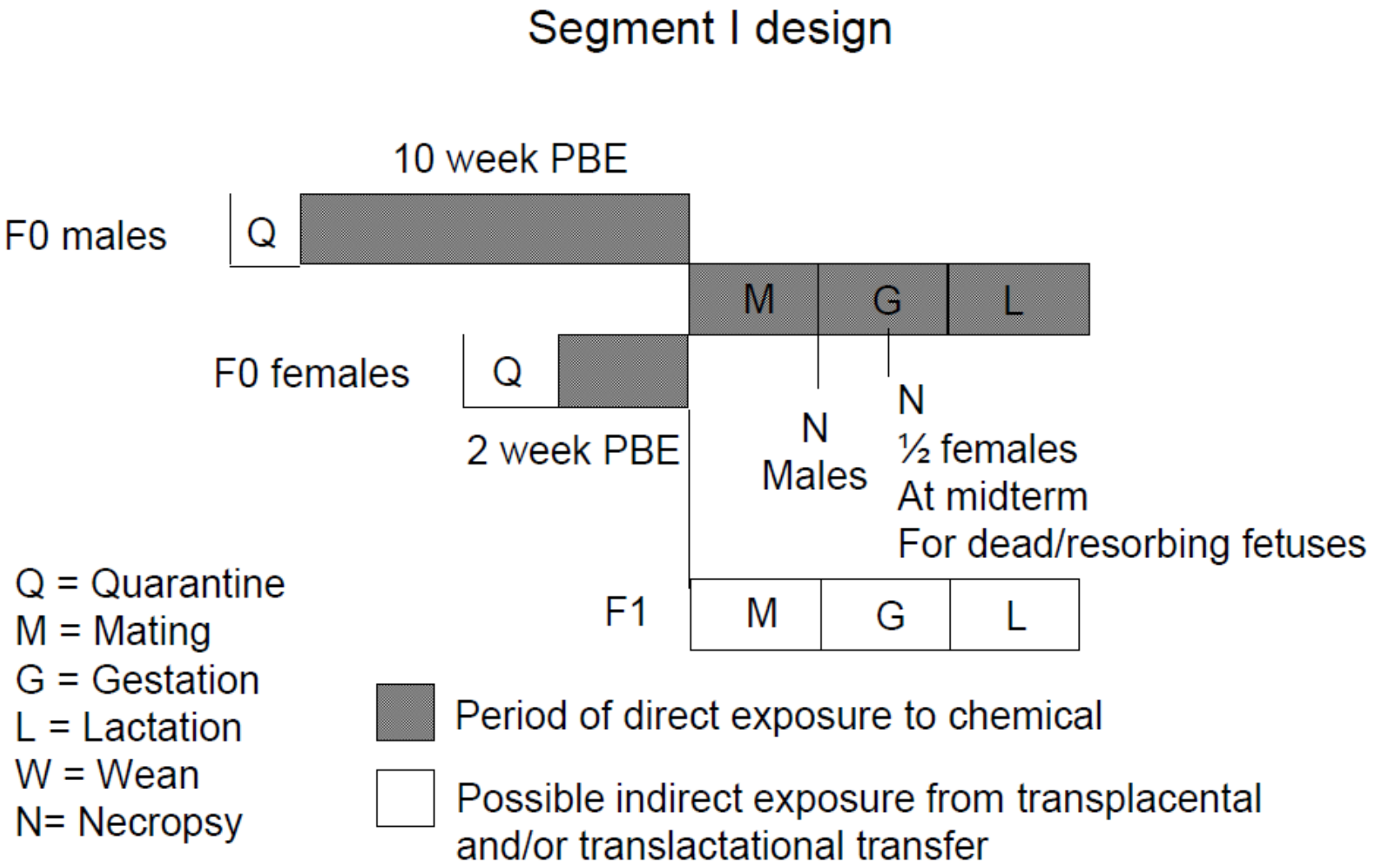
– Female rats treated for 14 days to cover three estrus cycles

– Three dose levels (without signs of maternal toxicity)

Segment I design



Studies may be combined



Endpoints in a Fertility Study

Males

- Testicular and epididymal weights*
- Testicular and epididymal histopathology*
- Sperm assessment*
- Mating behaviour and pre-coital intervals

Females

- Endpoints in a Fertility Study
- Oestrus cyclicity
- Ovarian weight and histopathology*
- Number of corpora lutea, implants, live and dead embryos
- Pre-coital interval

Index

- Fertility index = % matings that result in pregnancy
- Gestation index = % pregnancies yielding live litters
- Viability index = % animals surviving 4 days
- Lactation index = % of animals alive at 4 days that survive the 21 day lactation period
- Pup body weights pnd 4, 7, 14, and 21
- Gross necropsy and histopathology on some parents (both reproductive and non-reproductive organs)

Embryo-Foetal Development Study(segment II)

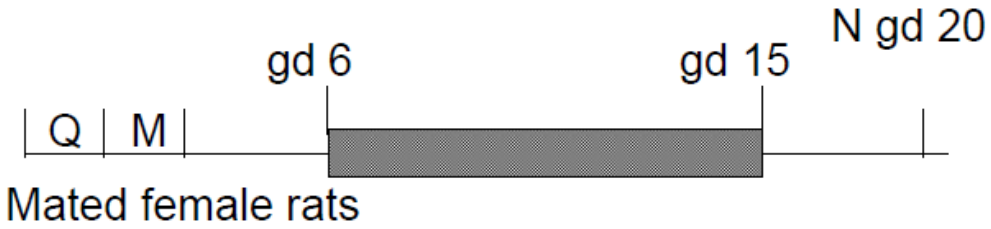
Usually conducted in 2 species: rat (20) and rabbit (12) at three dose levels

- Mated animals are treated during the period of organogenesis (days 6-18 in rabbits, 6-15 in rats)
- Pups delivered by Caesarean one day before expected parturition (21 days rat, 31 days rabbit)
- Uterus removed, weighed, and examined for dead or resorbed fetuses
- live pups are weighed, 1/2 examined for skeletal abnormalities, other half for soft tissue abnormalities histology

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

Segment II design



Mated female rats

Mated female rabbits

N= Necropsy
gd = gestational day

Direct exposure to pregnant dams

Endpoints in an embryo-foetal development study

DAM

- Clinical observations
- Weight gain & food consumption
- Number of implants and foetuses
- Post-implantation loss

FOETUS

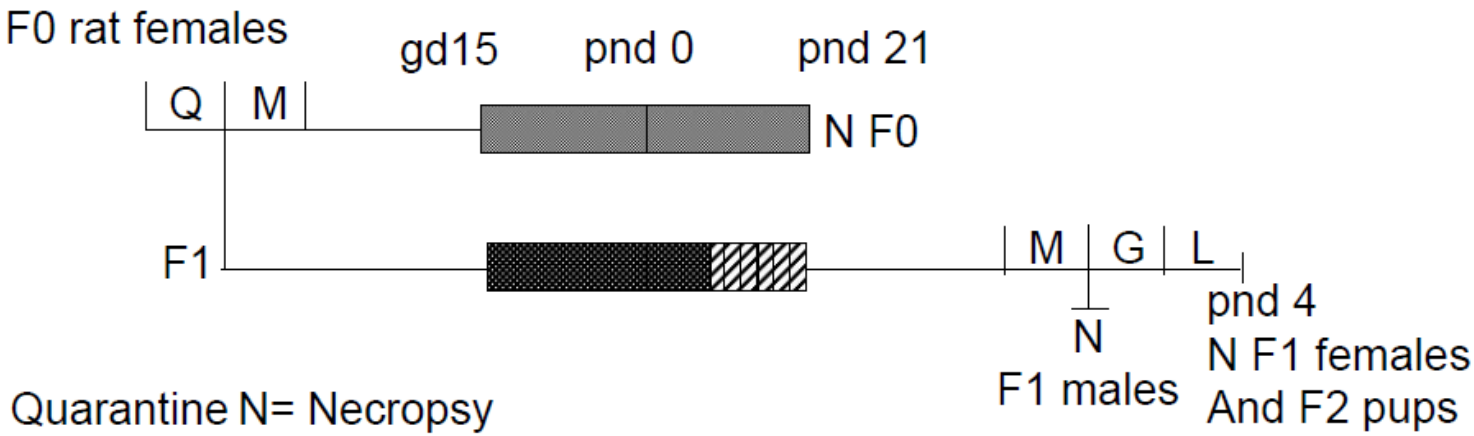
- Foetal & placental weights
- External abnormalities
- Soft tissue abnormalities
- Skeletal abnormalities
- Death & retarded development

Peri/postnatal study (segment III)

Usually conducted on one species (rats)

- n Pregnant females (20 per group)
- n 2-3 dosages administered from end of organogenesis (15 day) period through delivery and lactation
- Endpoints are: birthweight, survival, growth during first 3 weeks of life

Segment III design



Q = Quarantine N= Necropsy
M = Mating gd = gestational day
L = Lactation

Direct exposure to adults

Possible indirect exposure from transplacental and/or translactational transfer

Direct exposure to offspring if test material is administered via feed or water

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 (coat growth, locomotion, eyes open)

Behavioural development (motor activity, water maze

learning and memory, etc)

Reproductive performance of F1 (to mid-gestation)

Multigeneration study (segment IV)

Usually conducted on one species (rats)

- n F0 generation: 30 pairs M/F per dose
- at least 3 dose levels for 30-60 days
- prior to mating, continue exposure through the periods of gestation, birth, and development through the time of weaning, necropsy at pnd 150

- n Continue exposure of F1 generation (30 M/F pairs randomly selected) through mating, gestation, birth and postnatal development of F2 generation, necropsy at pnd 150
- n Repeat as above for F2 generation leading to F3

End-points in a multigenaration Study

- Fertility index = % matings that result in pregnancy
- Gestation index = % pregnancies yielding live litters
- Total number of litters
- Pup body weights and survival at pnd 4, 7, 14, and 21

Drug adverse effects on different stages of the cycle:

Pre-mating to conception

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

Implantation

- Diethylstilbestrol (hormone)
- Anti-histamines

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:

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

Sexual maturation

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