Joint BelTox-ETS DART course
"NAM based testing approaches"

Introduction to Developmental and Reproductive Toxicology (DART)

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DART studies are different

- Results provide risk assessment to the unborn foetus
- Repeat dose toxicity studies can identify testicular and ovarian lesions, but are unlikely to detect damage to reproductive function
- DART studies are designed to answer specific questions
- DART studies do not routinely include all the general (repeat dose) toxicity assessment parameters

What are we looking for?

- Adverse effects of treatment
- Dose relationships
- Evidence of different sensitivity of reproductive organ systems compared to other systems
- Differential effects in mother and foetuses/babies

What testing should we do?

DEPENDS ON THE PREDICTED EXPOSURE:

- Drugs tend to be given for short periods at high doses
- Target populations are well defined
- Usually given under surveillance

- Chemical exposures tend to be long term, or lifetime, at low dose levels
- All ages and subgroups exposed - multigenerational

Testing strategies - guidelines

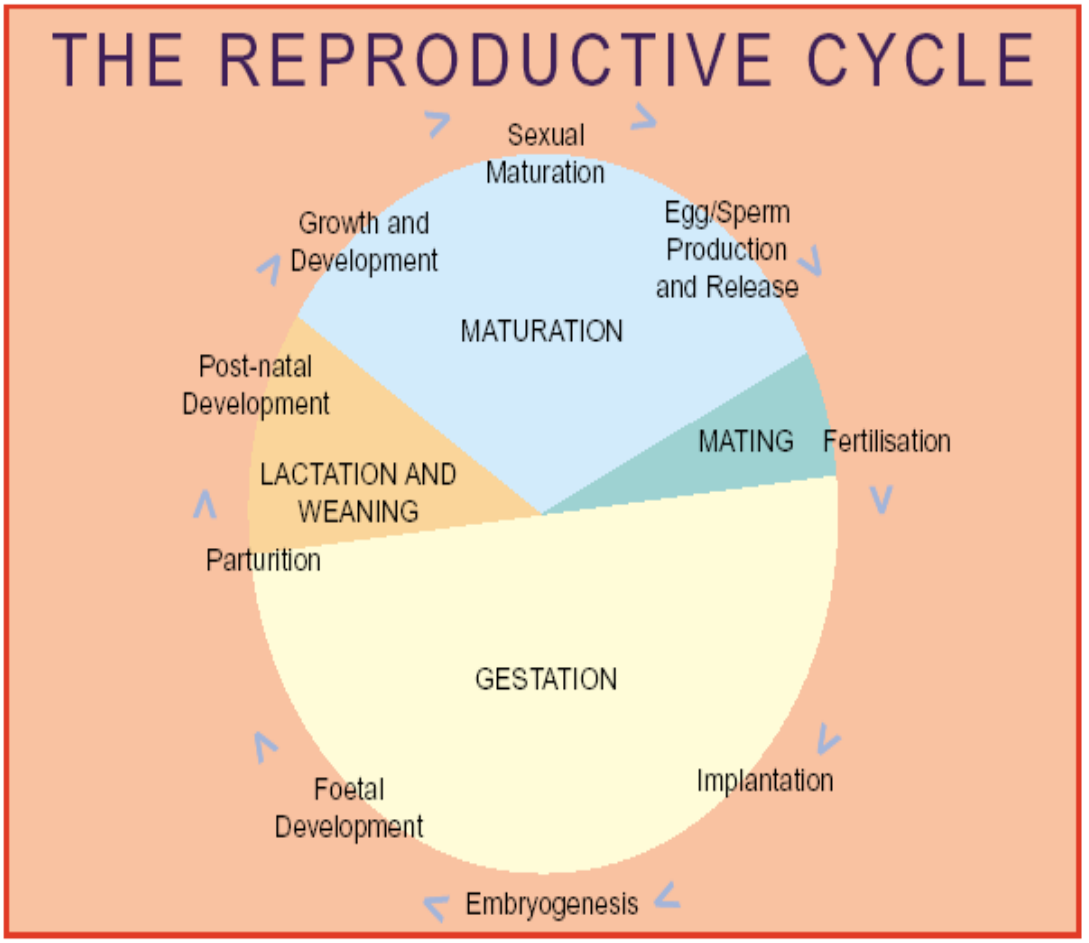
- PHARMACEUTICALS – ICH:
  - M3 (r2): Non-Clinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals
  - S5 (**r3**): Detection of Toxicity to Reproduction For Human Pharmaceuticals (**effective 30 July 2020**)

- CHEMICALS – OECD:
  - Test No. 414: Prenatal Development Toxicity Study
  - *Test No. 415: One-Generation Reproduction Toxicity Study*
  - *Test No. 416: Two-Generation Reproduction Toxicity Study*
  - Test No. 421: Reproduction/Developmental Toxicity Screening Test
  - Test No. 422: Combined Repeated Dose Toxicity Test with “
  - *Test No. 426: Developmental Neurotoxicity Study*
  - Test No. 443: Extended One-Generation Reproductive Toxicity Study

DART – Reproductive cycle

- ***Developmental Toxicity***
  - **In utero/at birth/early postnatal abnormal development (structural/functional)**

- ***Reproduction Toxicity***
  - **Sexual behaviour & fertility (♂ + ♀)**



PHARMACEUTICALS

Pharmaceuticals:
repro cycle subdivided in several studies

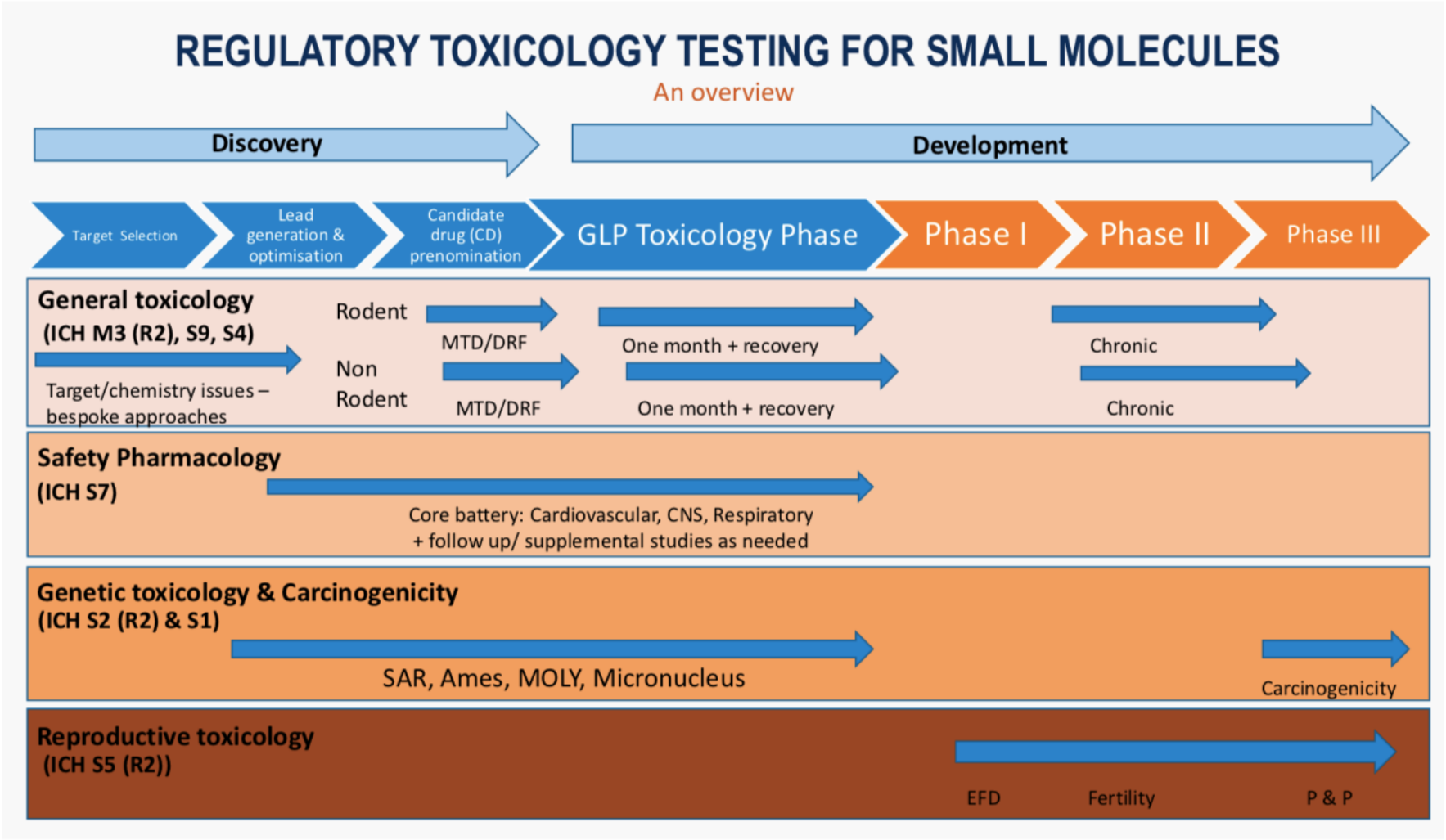
Fertility
- A - Pre-mating to conception - gametes, reproductive behaviour, fertilisation
- B - Conception to implantation

EFD
- C - Implantation to closure of hard palate - organogenesis

P&P
- D - Closure of hard palate to birth - organ maturation
- E - Birth to weaning - function maturation
- F - Weaning to sexual maturity

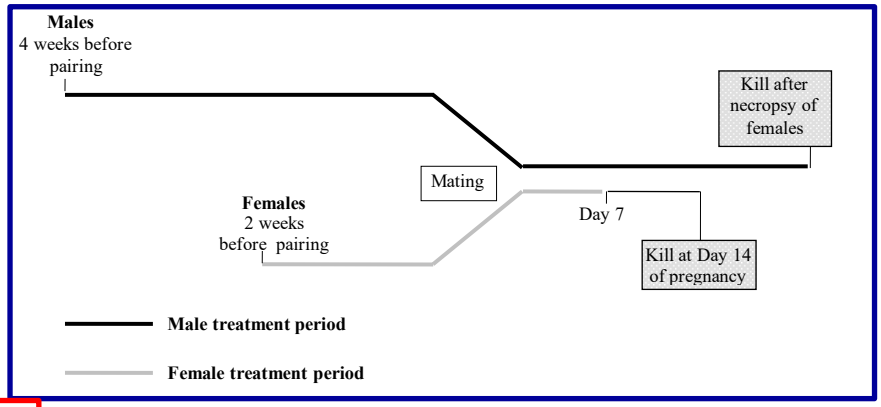
NOT ALL AT ONCE:
IN FUNCTION OF THE CLINICAL PHASE OF DRUG DEVELOPMENT

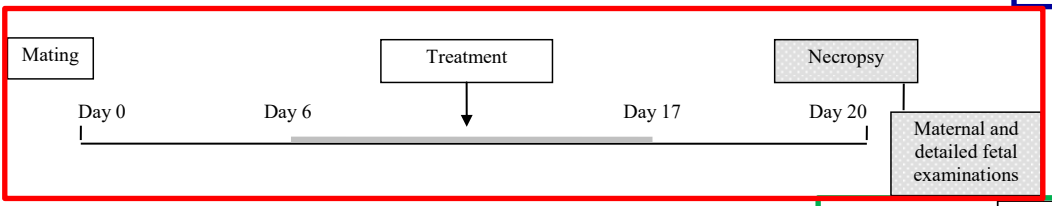
Pharmaceuticals: timing of studies



Pharmaceuticals: type of studies

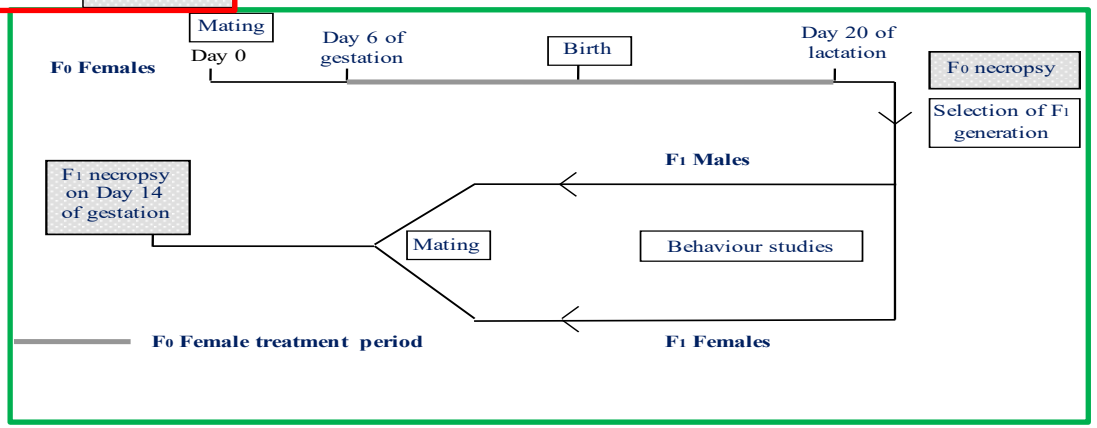
Fertility study
both sexes combined or separately





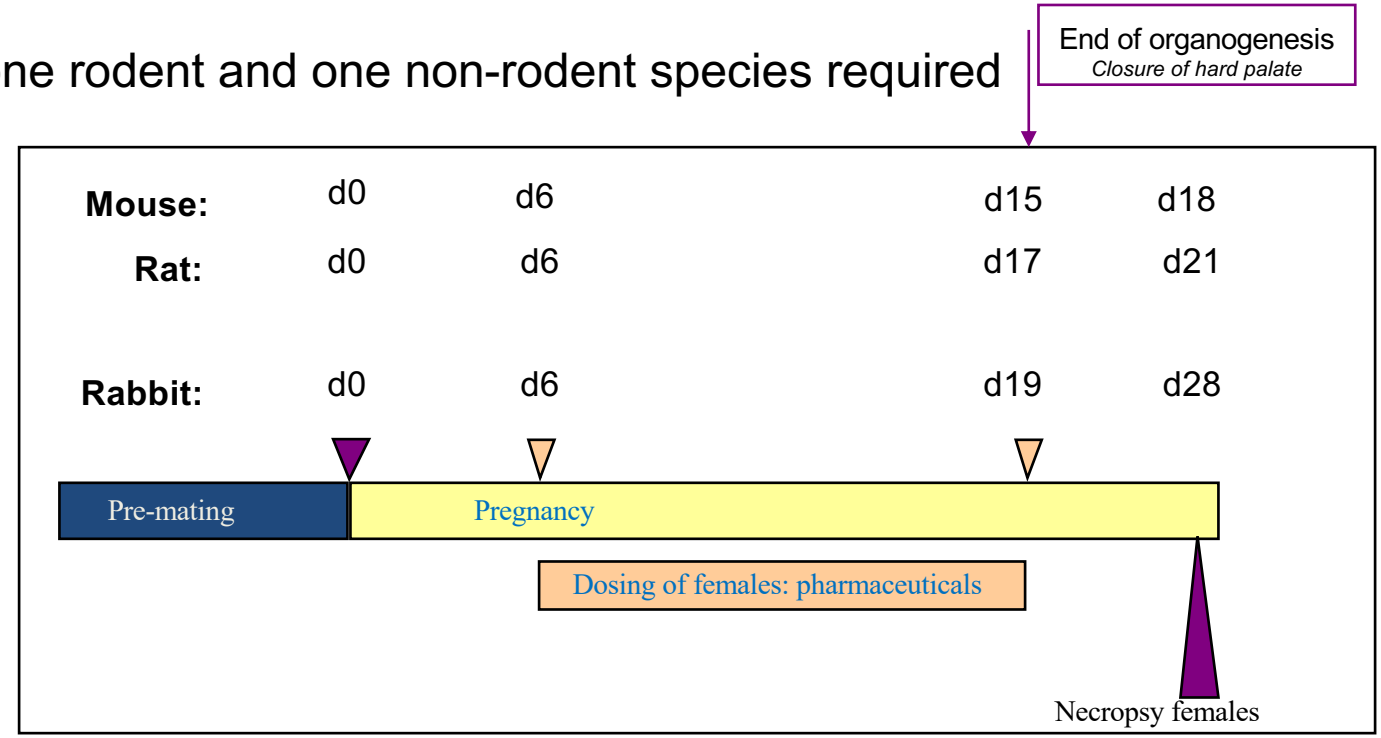
Embryofoetal development (EFD) study

Pre- and postnatal development study



EFD study

Generally one rodent and one non-rodent species required



Endpoints

Effects on pregnant dams
- Ovaria: n. corpora lutea
- Uterus: weight, implantations
  n. resorptions
  n. fetuses (live + dead)

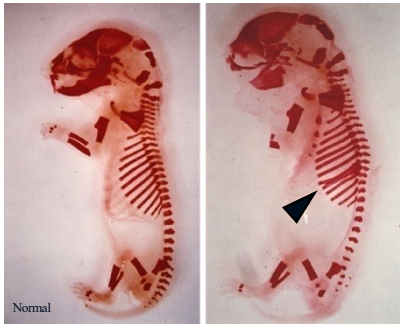
Effects on foetuses
- Macroscopic examination
- Visceral examination
- Skeletal examination

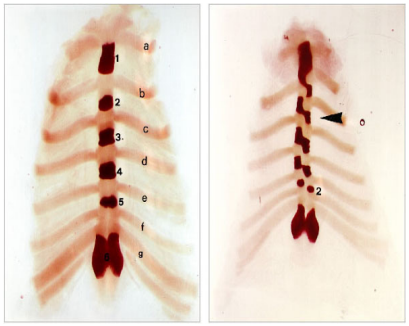
Foetal morphology

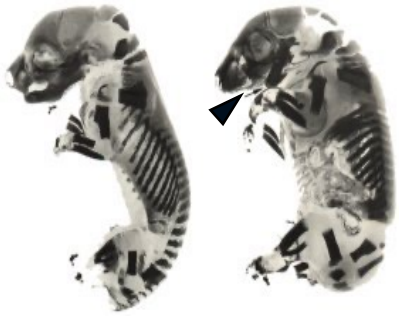
Effects on foetuses

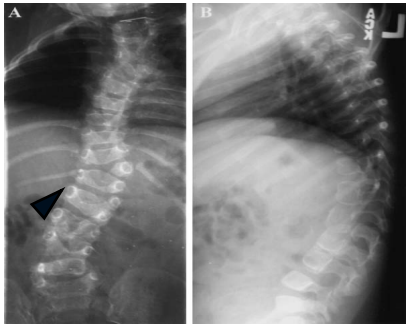
- Macroscopic examination
  - *ablepharia, agnatia, spina bifida, ….*
- Visceral examination (Wilson technique)
  - *Hernia diaphragmatica, expanded brain ventricles*
- Skeletal examination (alizarin-red technique, RX)
  - *Fused ribs, abnormal/fused vertebrae, scoliosis, polypodia, …..*

Minimum 16 litters/group to be evaluated!









EFD: still rodent and non-rodent species?

Critical Reviews in Toxicology

Taylor & Francis

ISSN: 1040-8444 (Print) 1547-6898 (Online) Journal homepage: https://www.tandfonline.com/loi/itxc20

Comparing rat and rabbit embryo-fetal developmental toxicity data for 379 pharmaceuticals: on systemic dose and developmental effects

Peter T. Theunissen, Sonia Beken, Bruce Beyer, William J. Breslin, Gregg D. Cappon, Connie L. Chen, Gary Chmielewski, Luc de Schaepdrijver, Brian Enright, Jennifer E. Foreman, Wafa Harrouk, Kok-Wah Hew, Alan M. Hoberman, Julia Y. Hui, Thomas B. Knudsen, Susan B. Laffan, Susan L. Makris, Matthew Martin, Mary Ellen McNerney, Christine L. Siezen, Dinesh J. Stanislaus, Jane Stewart, Kary E. Thompson, Belen Tornesi, Jan Willem Van der Laan, Gerhard F. Weinbauer, Sandra Wood & Aldert H. Piersma

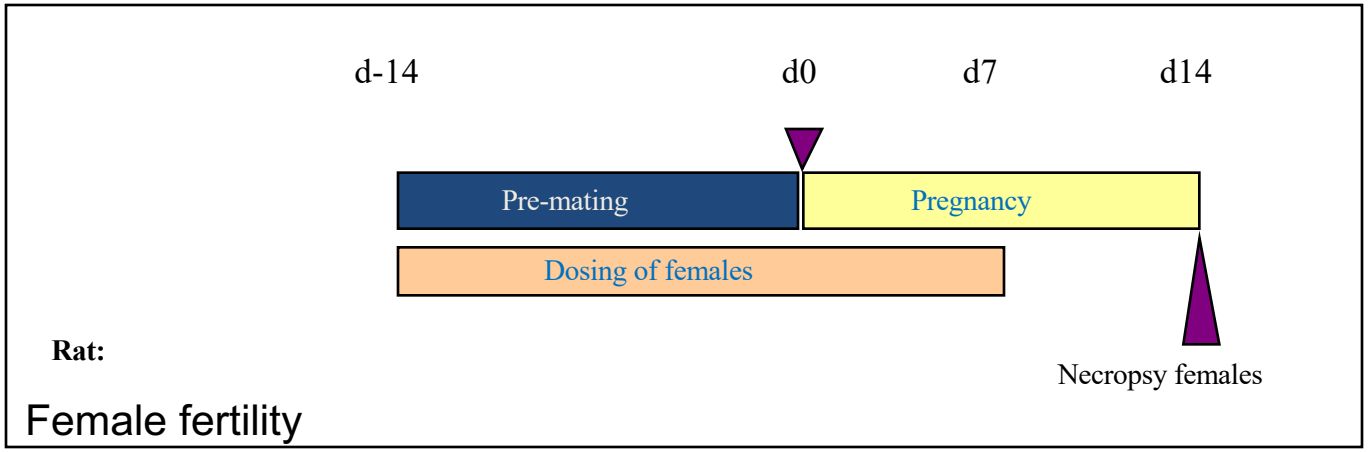
Also: Braakhuis et al. 2019; doi: 10.1016/j.yrtph.2019.104410

Fertility study: general aspects

- Oestrous cycles and mating behaviour
- Implantations
- Early embryonic survival
- Male reproductive organ weights
- (CASA – computer assisted sperm assessment)

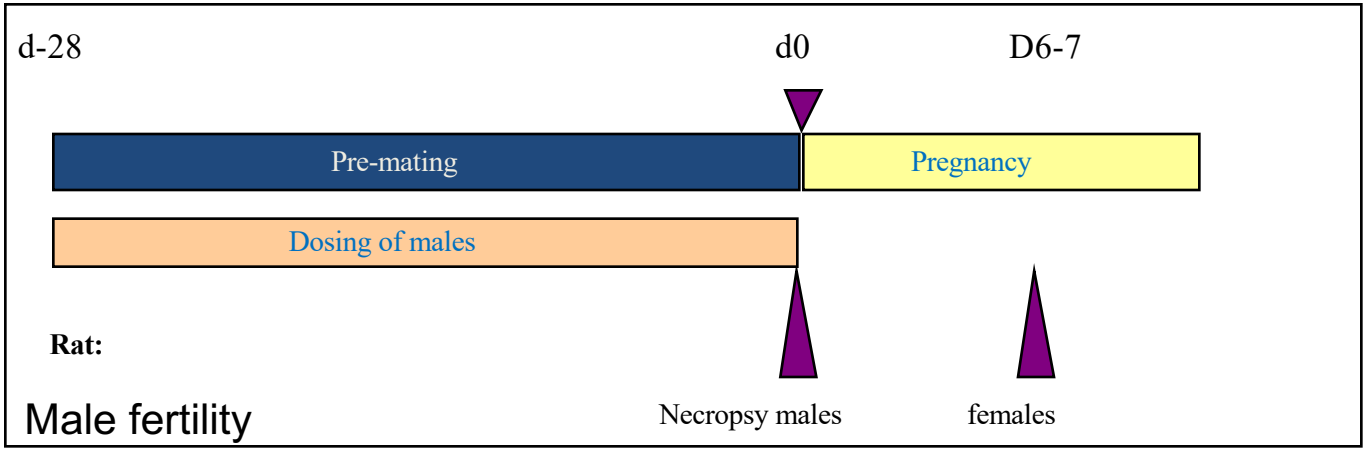
Male and female fertility study: design

Endpoints



Implantations
Resorptions
Live/dead embryos

Minimum 16/group/sex



Sperm count
Sperm motility
Sperm morphology
Implantations
Live/dead embryos

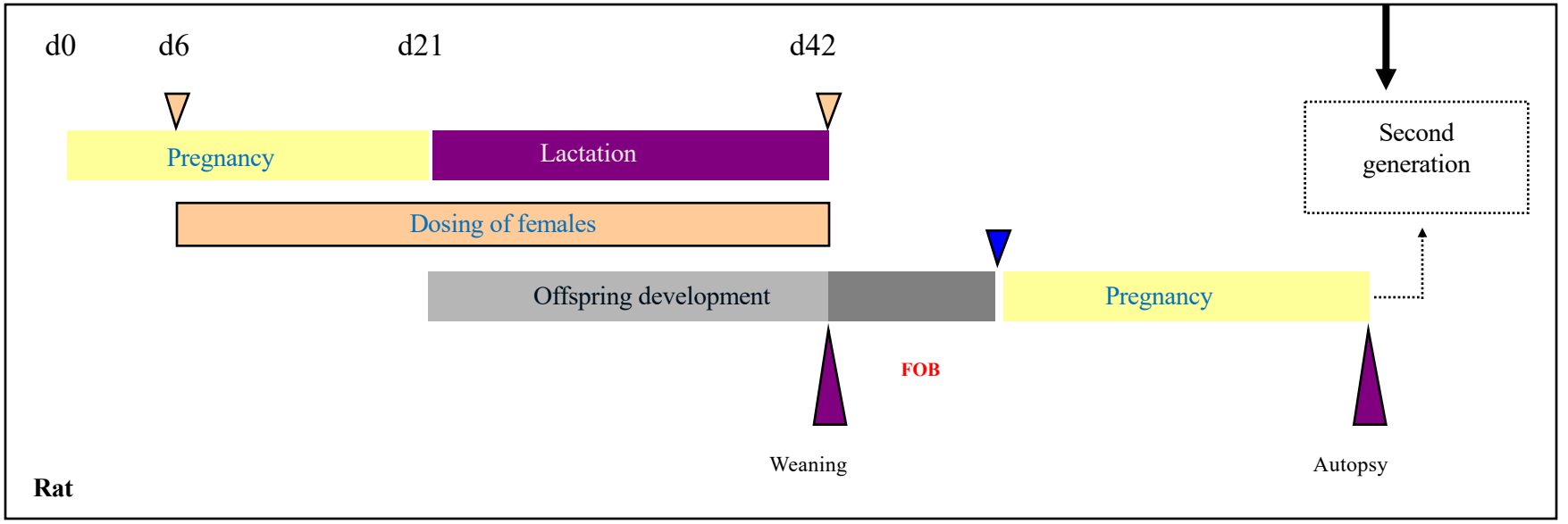
Fertility: calculated parameters

- Copulation index = $\frac{\text{number of animals mated}}{\text{number of animals paired}}$ x 100
- *Fecundity index*
- Fertility index = $\frac{\text{number of animals pregnant}}{\text{number of animals paired}}$ x 100
- Pre-implantation loss (%) = (a - b)/a x 100
- Post-implantation loss (%) = (b - c)/b x 100

where:
a = number of corpora lutea
b = total number of implantation sites
c = number of live foetuses

Pre- and postnatal development study

Not for pharmaceuticals



Endpoints

Minimum 16 litters/group to be evaluated!

Effects on pregnant dams
- General health status
- Fertility, pregnancy, birth

Effects on 1st generation offspring
- Survival and development
- Behaviour, motility, reflexes, vital senses → **FOB**
- Reproductive potential / capacity

enhanced Pre- and Postnatal Development (ePPND) study in NHPs

- Mainly for biopharmaceuticals
- Combination of EFD and PPND endpoints
- Approx. 16 pregnant females/group and dosing is extended throughout gestation to parturition
- Offspring monitored until at least 28 days of age (BW together with physical and/or functional assessment and FOB)
- Mother-infant interactions + offspring systemic drug levels assessed
- Necropsy after PND28, including structural examinations

CHEMICALS

Chemicals: type of studies

Table R.7.6-1 Summary of information requirements for reproductive toxicity in REACH (Annexes VII to X).

Study	Annex VII (<10 t/yr)	Annex VIII (≥10 t/yr)	Annex IX (≥100 t/yr)	Annex X (≥1000 t/yr)
Screening test for reproductive /developmental toxicity (OECD TGs 421 or 422)		Required. If a prenatal developmental toxicity study is available or proposed, it is strongly recommended to consider conducting a screening study in addition to the prenatal developmental toxicity[1] study. If an extended one-generation reproductive toxicity study is available or is proposed, a screening study may not need to be conducted.	Strongly recommended if no higher tier study (such as OECD TG 443) is/will be available to address fertility and peri/post natal development	(a higher tier study is required)
Prenatal developmental toxicity study (EU B.31, OECD TG 414)		May be proposed in cases of serious concern[2] for prenatal developmental toxicity instead of the screening study.	Required in one species; second species may be triggered[3]	Required in two species
Extended one-generation reproductive toxicity study (EU B.56, OECD TG 443)[4]		May be proposed in cases of serious concern for fertility instead of the screening study[2]	Required in one species if triggered[5]; second species/strain may be triggered in exceptional cases	Required in one species unless already conducted at previous Annex level; second species/strain may be triggered in exceptional cases

Guidance on Information Requirements and Chemical Safety Assessment

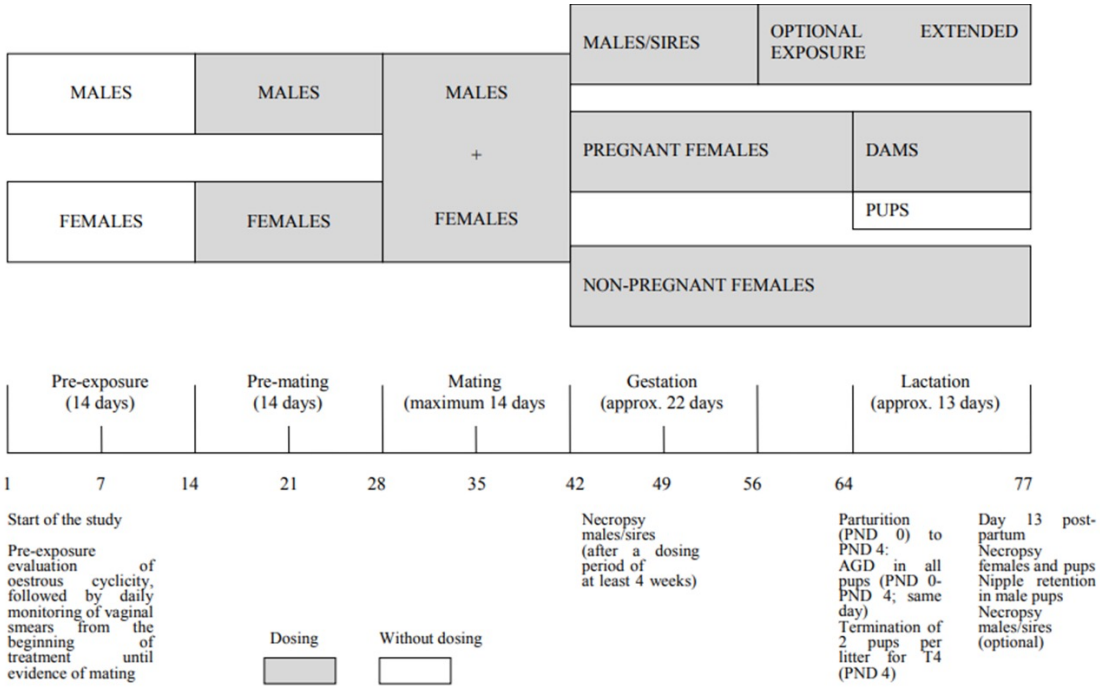
Chapter R.7a: Endpoint specific guidance

Version 6.0

July 2017

Reproduction/development toxicity screening test study (TG421)

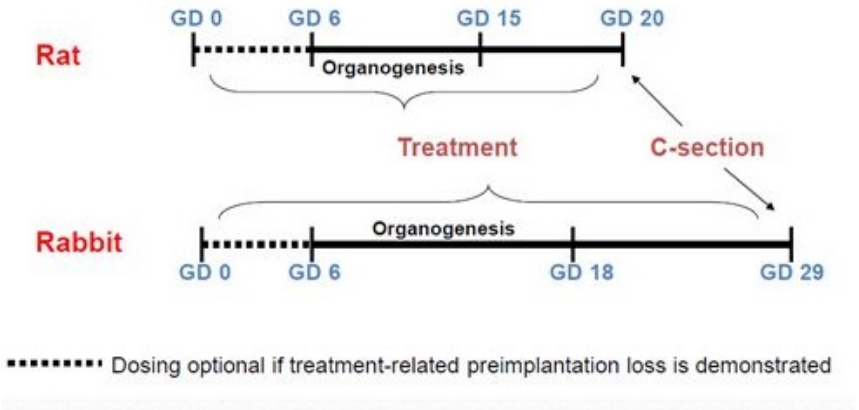
- N=10/group/sex
- TG422 has additional 28-day tox endpoints
- Range finding study for generational studies
- Gavage or dietary route



Exposure period	Endpoints in parental and/or offspring
From 2 weeks prior to mating until day 13 of lactation	• Fertility • Pregnancy length and birth outcome • Histopathology of sex organs and target organs (and brain in TG 422) • Foetal and pup growth and survival until day 13 of age • Endocrine disruption endpoints • FOB (TG 422) • Clinical pathology (TG 422)

Prenatal Development Toxicity Study (TG414)

Exposure period	Endpoints in parental and/or offspring
From implantation to the day before birth	• Litter composition (e.g. Resorptions, live, dead foetuses) • Embryonic development • Foetal growth • Morphological variations and malformations • Thyroid evaluations



Key differences with pharmaceutical EFD

- Two species not always required
- Exposure through end of gestation
- ED endpoints incorporated (thyroid weight, thyroid hormones in dams and AGD in foetuses)
- Recommended 20 pregnant females at necropsy
- Preference for bone and cartilage examination

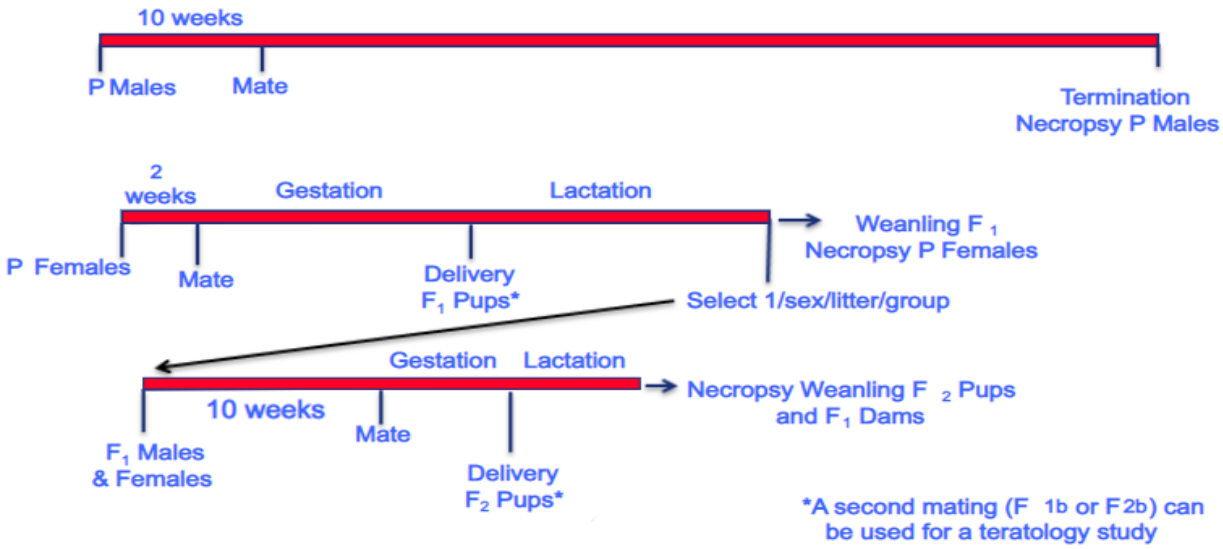
Generational studies

Exposure period	Endpoints in parental and/or offspring	Guideline(s)
Continuously over one, two or several generations	• Growth, development and viability • Pregnancy length and birth outcome • Histopathology of sex organs and target organs • Fertility • Oestrus cyclicity and sperm quality • Endocrine disruption endpoints (TG 443) • Developmental neurotox and immunotox option (TG 443)	*TG 415: One Generation study (discontinued)* TG 416: Two Generation study TG 443: Extended One-Generation Reproductive Toxicity Study

Key differences with pharmaceutical PPND

- Continuous exposure in parents and offspring for longer period
- Histopath routine, including detailed examination of testes and ovaries (including follicle count)
- Sperm analysis
- Physical developmental endpoints not mandatory, except for sexual maturation
- Typically dietary route of administration

2-generation reproduction toxicity study (TG416)



Extended one generation reproduction toxicity study (EOGRTS - TG443)

See presentation Emily Richmond

Chemicals: other studies

TG 426 Developmental Neurotoxicity:

- Required for certain chemicals but not a core data requirement
- 20 dams/group exposed through gestation and lactation
- Evaluates neurotoxicity in offspring after PPN exposure (n=10-20 depending on endpoint)

Age Periods / Endpoints	Pre-weaning (b)	Adolescence (b)	Young adults (b)
Physical and developmental landmarks			
Body weight and Clinical Observations	weekly (c)	at least every two weeks	at least every two weeks
Brain weight	PND 22 (d)		at termination
Neuropathology	PND 22 (d)		at termination
Sexual maturation	--	as appropriate	--
Other developmemental landmarks (e)	as appropriate	--	--
Functional/behavioural endpoints			
Behavioural ontogeny	At least two measures		
Motor activity (including habituation)	1–3 times (f)	--	once
Motor and sensory function	--	once	once
Learning and memory	--	once	once

CONCLUSIONS

- DART studies aim to predict effects of pharmaceuticals and chemicals on the reproduction ability of human beings by assessing effects in animals

- The total process of reproduction is divided into a series of manageable studies to provide answers to specific challenges to the reproductive processes

- Approaches for chemicals and pharmaceuticals are different

WANT TO LEARN MORE ABOUT DART TESTING?
VISIT ETSOC.COM AND SUBSCRIBE FOR THE 2026 ETS DART COURSE