Reproductive Toxicity assessment under REACH

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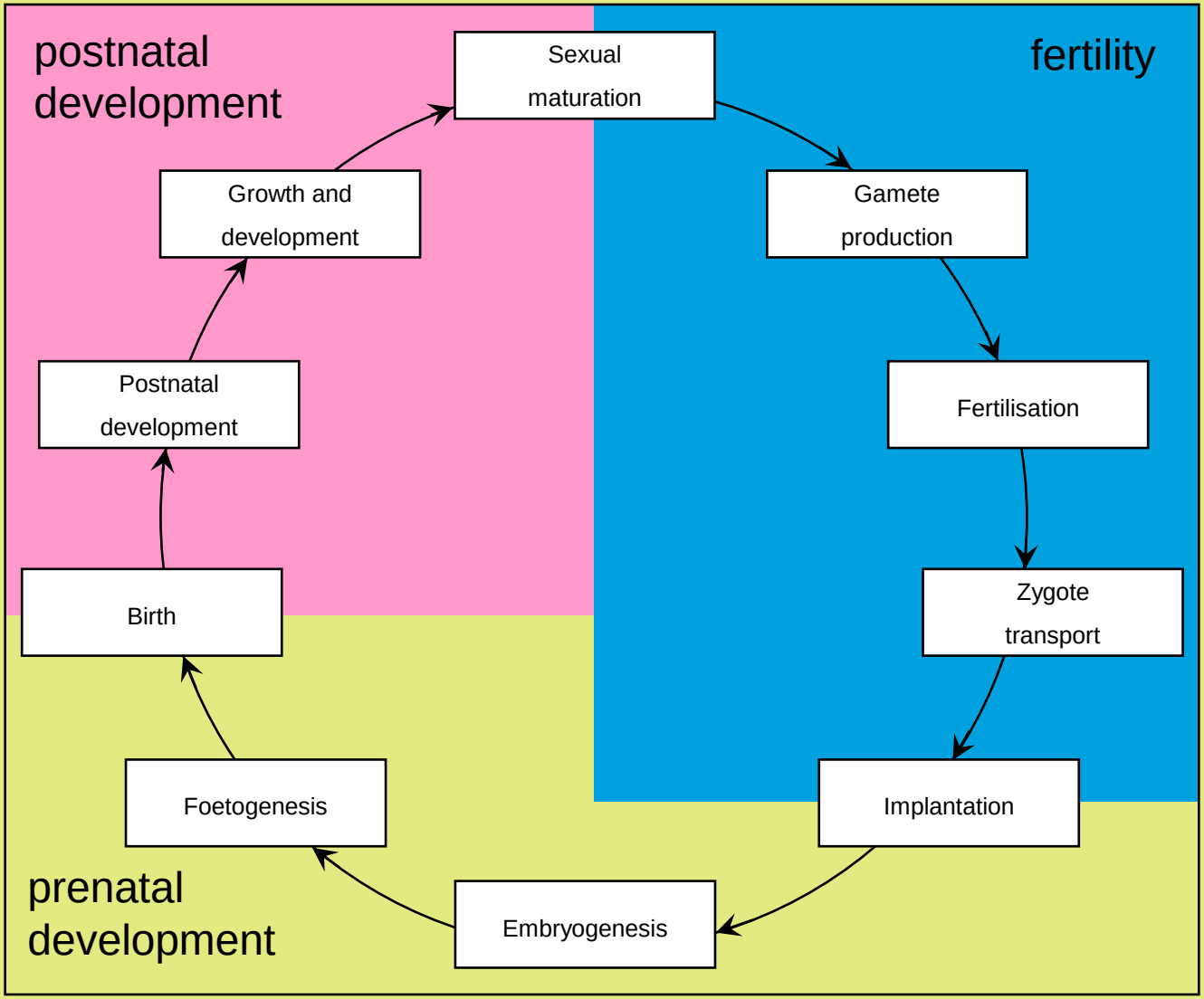
The Reproductive Cycle



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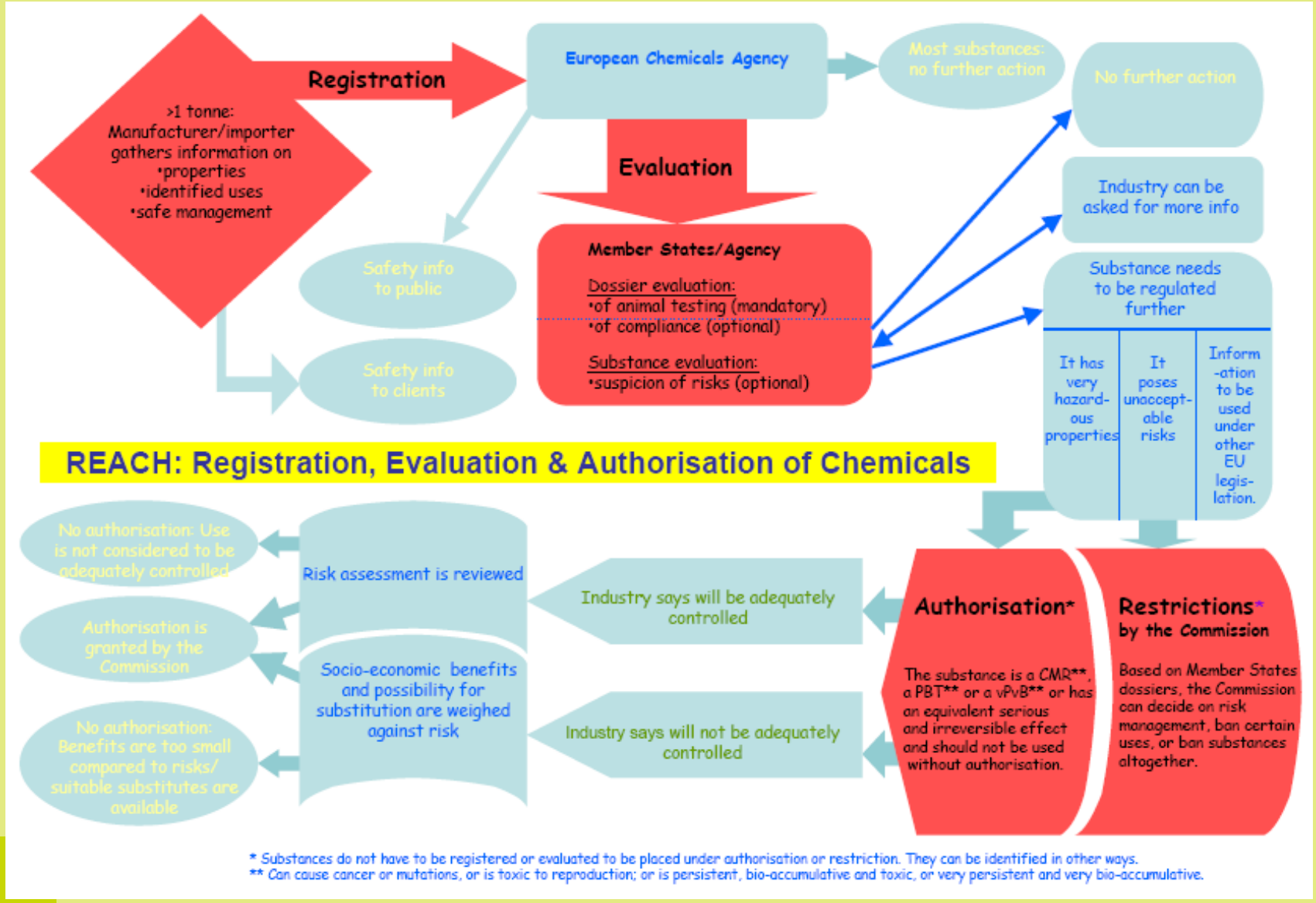
Characteristics of reproductive toxicity

- Reproductive effects are very diverse
  - (cf. sperm quality, malformations, behaviour)
- Type and severity of effects depend on the exposure window within the reproductive cycle
- Effects may become obvious during exposure or much later in the cycle
- Type and severity of effects observed depend on the moment of effect assessment
- Reproductive effects may be caused directly or indirectly via parental toxicity

Aims of reproductive toxicity testing

- Classification and labelling
  - On the basis of specific reproductive toxicity
  - May arise from general tox testing or from dedicated reproductive toxicity testing
  - Requires testing reproductive end points
- Risk assessment
  - On the basis of most sensitive parameter
  - Need not always be a reproductive parameter
  - Requires testing reproductive end points

REACH



* Substances do not have to be registered or evaluated to be placed under authorisation or restriction. They can be identified in other ways.
** Can cause cancer or mutations, or is toxic to reproduction; or is persistent, bio-accumulative and toxic, or very persistent and very bio-accumulative.

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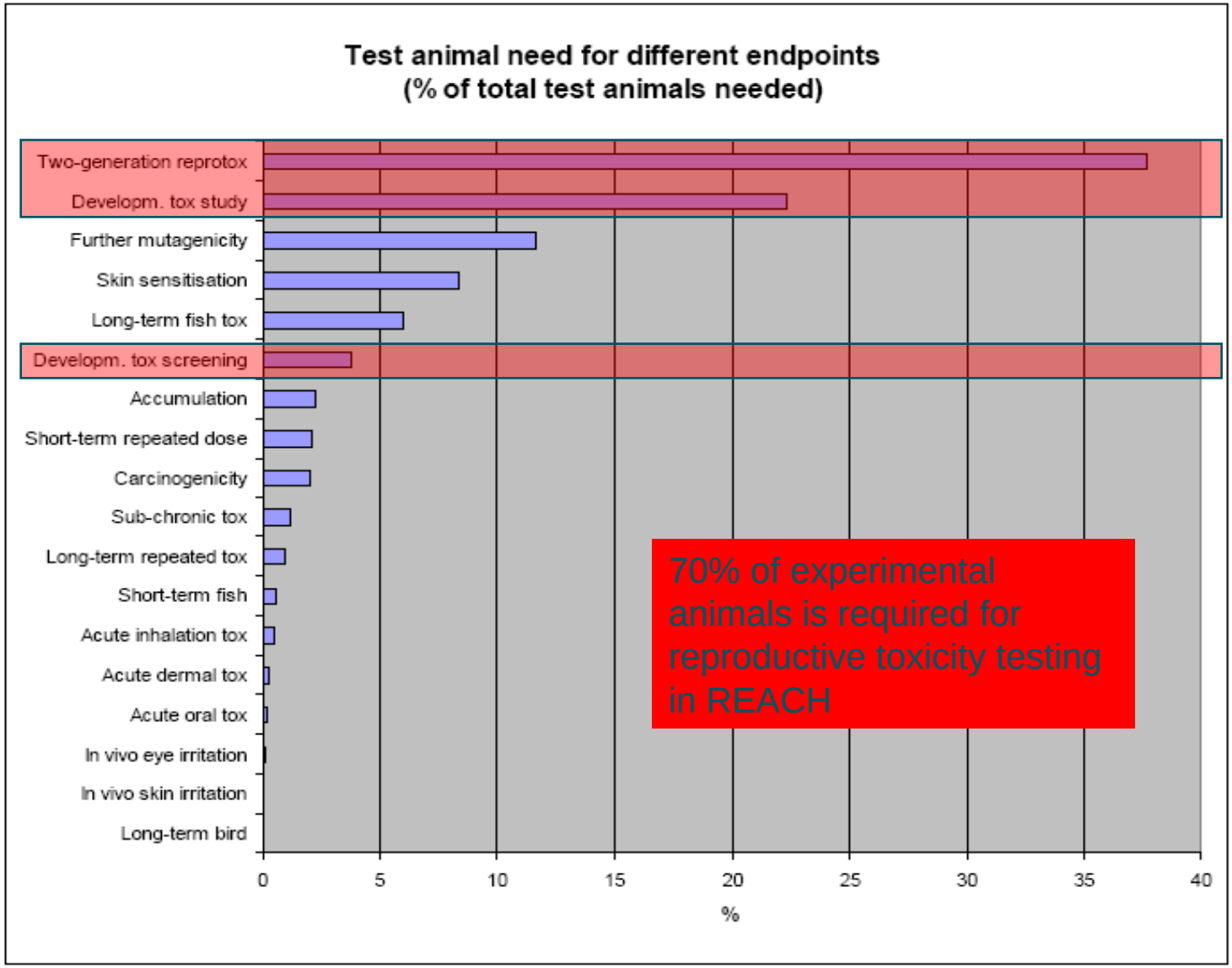

Van der Jagt et al., ECB 2004

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Available protocolized in vivo test systems

- Two-generation study (OECD416) (P0 prem.d28- F2 pnd21)
- Developmental toxicity study (OECD414) (gd6-20)
- Reproductive toxicity screen (OECD421) (prem.d14-pnd6)
- Developmental neurotoxicity test (OECD426) (gd6-pnd21)
- Enhanced 28-day subchronic toxicity test (OECD407)
- Uterotrophic assay (OECD draft)
- Hershberger assay (OECD validation effort)
- Extended one-generation study (OECD task force)

- OECD GD34 guidance: validation of new methods
- OECD GD43 guidance: reproductive toxicity testing strategy

OECD407 enhanced: additional end points

Organ/tissue weights
1. Testes (each weighed separately)
2. Seminal vesicles + coagulating glands
3. Prostate (possible dissection and separate weights for ventral and dorsolateral prostate), ovaries
4. Thyroid
5. Uterus

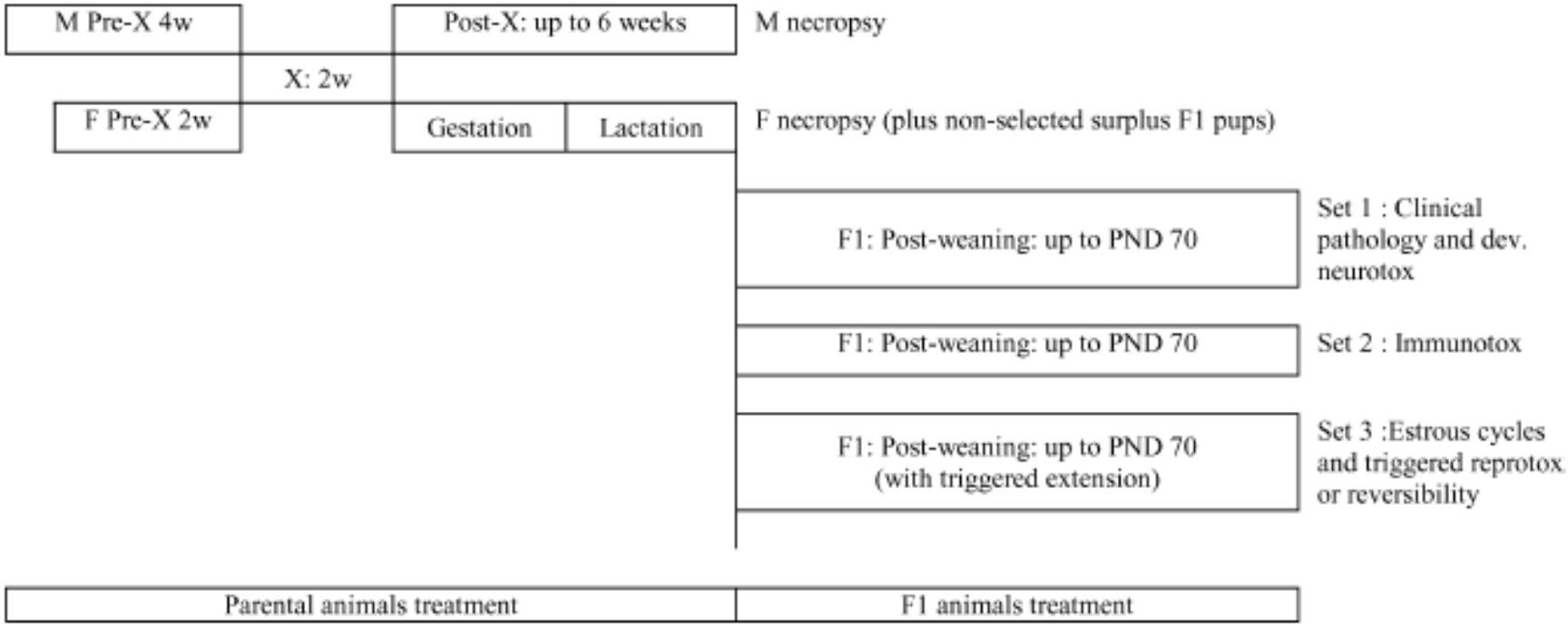
Histopathology
1. Pituitary
2. Vagina
3. Epididymides, seminal vesicles + coagulation glands
4. Mammary gland

Thyroid hormones
1. Circulating levels of T3 and T4
2. Circulating levels of TSH

Spermatology
1. Epididymal sperm number
2. Sperm morphology

Estrous cycle
Daily vaginal smears to assess estrous cycling via epithelial cytology for at least 5 days to ensure necropsy during diestrus

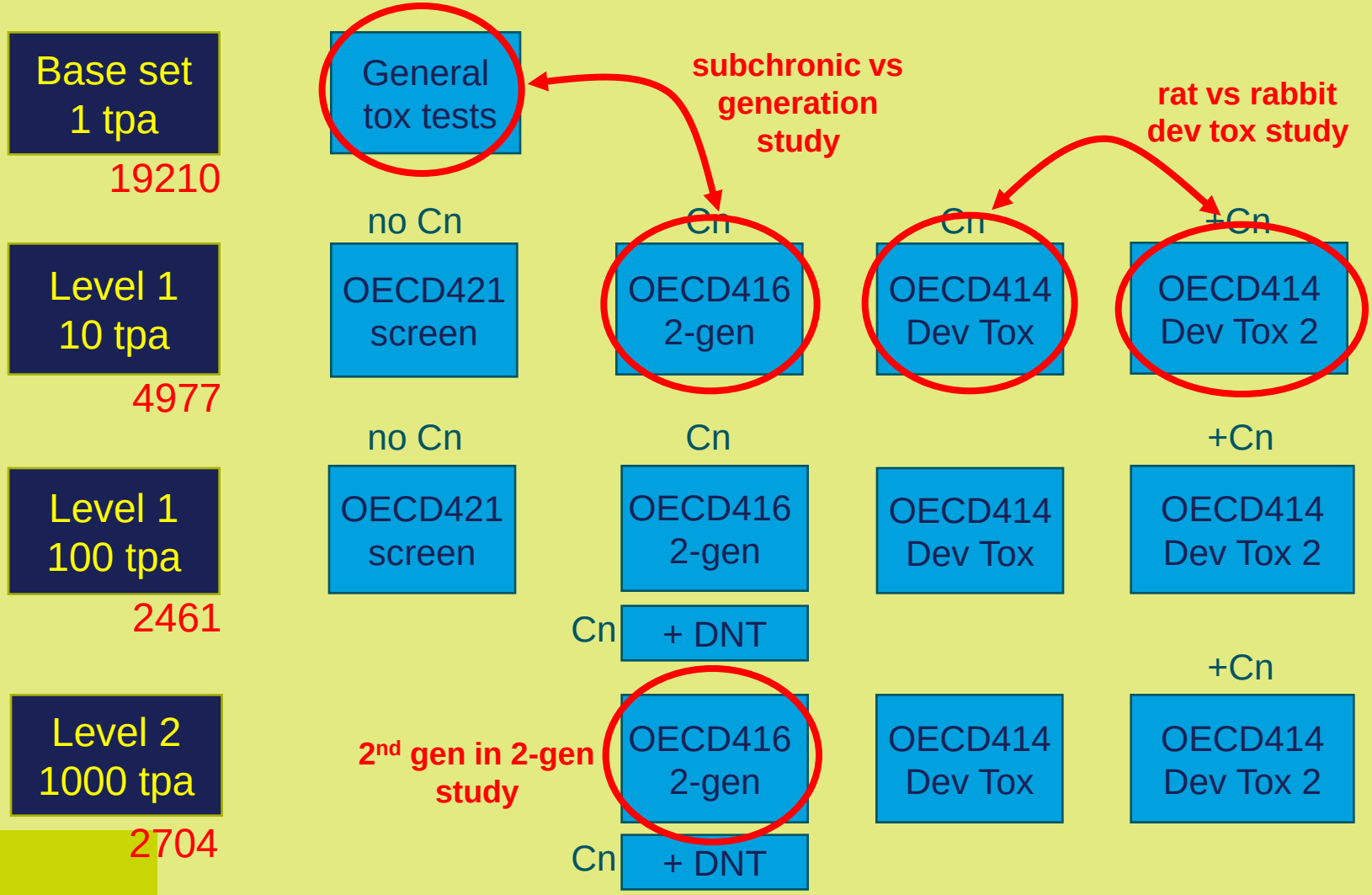
Extended one-generation study protocol



Cooper et al., 2006: Crit Rev Tox 36: 69-98.

EU REACH reproductive toxicity testing strategy

(+ REACH numbers)



No reprotox testing for: 1. genotoxic carc / mut , 2. in case of low tox and no systemic absorption or no sign. human exposure

Retrospective analyses of existing data

(Can hazard assessment be simplified by changing the testing strategy?)

- Impact of the second generation in the 2-generation study

  (Janer et al., Reprod. Toxicol. 24: 97-102 (2007))

- Comparison of NOELs and critical end points in subchronic versus 2-generation study in the rat

  (Janer et al., Reprod. Toxicol. 24: 103-113 (2007))

- Comparison of rat and rabbit developmental toxicity studies

  (Janer et al., Regul. Toxicol. Pharmacol. 50(2):206-17 (2008))

Methods

- Substances classified as toxic to fertility or to development according to Directive 92/32/EEC or California EPA
- Available subchronic and two-generation studies, carried out according to or close to OECD guidelines
- Historic data interpretation taken from existing peer reviewed risk assessment reports

Subchronic study: C&L

n=36 subchronic studies, 30 fertility toxic substances

**** Showed clear fertility related toxic effects**:

- testicular toxicity in all studies, except for one that showed ovarian toxicity

**** Showed alerts of toxicity to fertility** :

- changes in reproductive organ weights or hormone levels

**** Did not show fertility toxic effects**:

- did not include histopathology of reproductive organs
- the highest dose tested was similar to the lowest dose inducing effects in the two-generation study

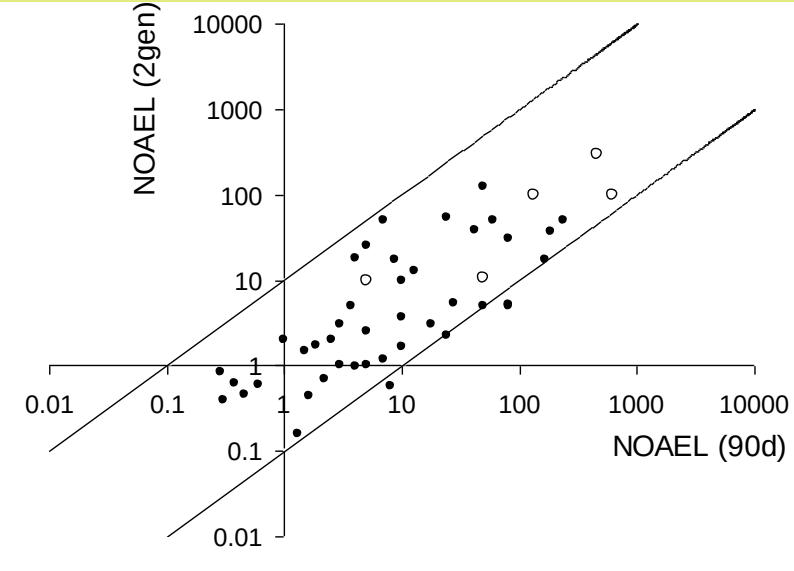
The subchronic study did not always detect toxicity to fertility → the two-generation studies had an impact on C&L

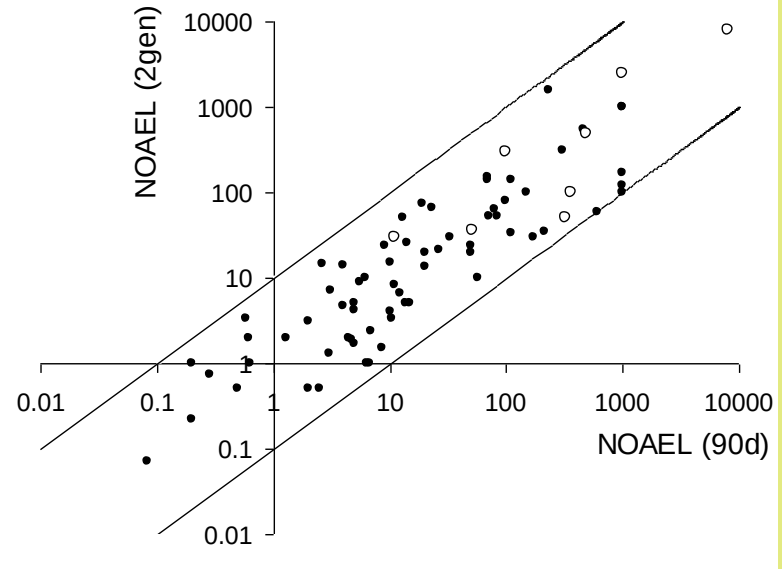
Two-generation vs. Subchronic study: NOAEL

Classified for reproductive toxicity (n = 47)

Not classified for reproductive toxicity (n = 75)

- mg/kg bw/day
- ppm





The two generation and the subchronic toxicity tests led to similar overall NOAELs

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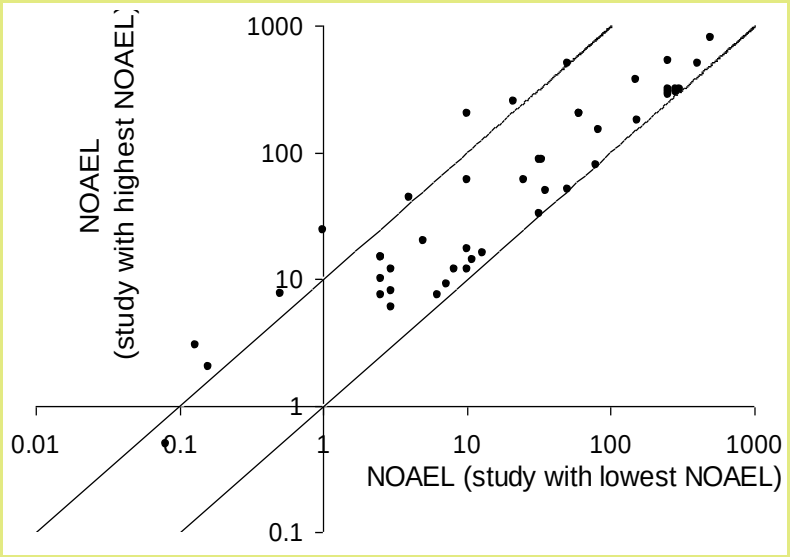
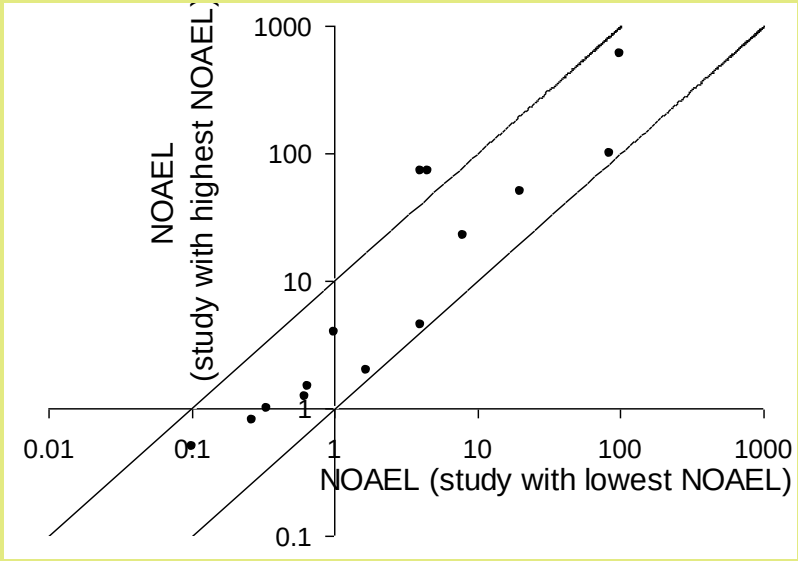
‘Replicated’ studies: NOAEL

Subchronic studies
(36 substances, 77 studies)



Two-generation studies
(12 substances, 25 studies)



Replicate studies with the same compound showed the same scatters in NOAELs as the comparison between study types

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Impact on C&L and NOAEL of omitting the 2nd gen in a 2-gen reproductive toxicity study ?

Classification & Labelling

- In 3 out of 176 studies, reproductive toxicity was observed in F2 offspring and not in F1 offspring. However, the effects were not considered severe enough (in the existing risk assessment reports) to justify classification for reproductive toxicity.

NOAEL

- In 2 out of 176 studies the NOAEL of the study depended on the effects on F2 offspring. However:
    - Triadimefon: No effects were found on the F3 offspring
    - Vinclozolin: Reduced epididymal weight was considered as a marginal effect in the JMPR evaluation
    - NOAELs in both cases only 6-fold lower in F2 than in F1 offspring

The second generation neither had an impact on overall NOAEL nor on Classification and Labelling

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Impact of leaving out the F1 adults?
(i.e., F1 maintained until weaning only)

Impact on Classification and Labelling

- In 6 studies (5 substances) out of 176 studies (58 on reproductive toxic substances) showed reproductive toxic effects on F1 adults, but not on P0 adults. Most of these substances were phthalates.

Impact on NOAELs

- In 6 out of 176 studies the NOAEL of the study depended on the effects on F1 adults. In these studies, the effects on F1 occurred at doses 2- to 10-fold lower than in P0 adults.

In some studies, findings in the F1 adults had an impact on NOAELs and on Classification and Labelling

Discussion

- These retrospective analyses support the development of an extended 1-generation study, however, these analyses may be biased by the availability of data
- A comprehensive retrospective data analysis of 2-generation study results is advised to optimize the design of the extended one-generation study and its use in the strategy for reproductive toxicity testing
- The fundamental choice is between **principle** (always test all possible end points within the reproductive cycle to exclude missing any effect) and **pragmatism** (save 1200/2600 animals by excluding 2nd generation end points, which are highly unlikely to contibute to RA or C&L)