EXTENDED ONE-GENERATION REPRODUCTIVE TOXICITY STUDY (EOGRTS) – CONCEPT & DESIGN

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REPROTOXICITY TESTING & THE EOGRTS

- Reproductive toxicity testing in EU
- EOGRTS
  - What is it ?
  - Why do we want/need it ?
- What are the issues ?
  - Theoretical
  - Practical
- Interpretation of legal text (guidance)
- Questions

REPROTOXICITY TESTING IN THE EU

- REACH was the **first** chemicals regulation in the world that asks for reprotoxicity data as a **standard requirement**
- Prior to REACH, only substances that were suspected (e.g. by structural alert or from indications from toxicity studies or epidemiology) were tested and substances designed to have a biological effect
- REACH (EC Reg 1907/2006): reprotoxicity testing required based on **production volume**:
  - ≥ 10 t/a: a reproduction/developmental toxicity screening in one species (OECD 421 or OECD 422)
  - ≥ 100 t/a: a prenatal development in one species (OECD 414) and, if triggered, two-generation study in one species (OECD 416)
  - ≥ 1000 t/a: a prenatal development in a second species (OECD 414) and a two-generation study in one species (OECD 416)

REACH 'IN ACTION'

- For the REACH **2010 registrations** (i.e. high volume and CMR substances), **automatically** an OECD 416 was triggered (i.e. 2-generation reproductive toxicity study)
- However, toxicity testing required by the higher Annexes, which involves **vertebrate species,** cannot be conducted without permission from ECHA → Registrant is required to submit a **Testing Proposal**
- Testing Proposals are public and ECHA, Member States and Stakeholders can comment
- For **all** Testing Proposals with an OECD 416, no exceptions, animal activist organisations and some Member States (!) suggested to substitute the OECD 416 with an EOGRTS
- However, the EOGRTS was not a valid test under REACH
- Push to change REACH legislation….

AMENDMENT OF REACH (EC REG 2015/282)

- Prework: EC Reg 900/2014, Amendment to Technical Progress: including the EOGRTS as an 'acceptable' EU method (B56), along with a series of tests for developmental neurotoxicity and for ED
- Amendment of Annexes VIII, IX and X as per 20/02/2015:
  - The two-generation reproductive toxicity test (OECD 416) is replaced by the EOGRTS (OECD 443)
  - Existing two-generation reproductive toxicity test results (studies initiated before regulation came into force) will fulfil the information requirement
  - Requirement for ECHA to define 'rules for the adaptation of the information requirements' for the additional cohorts, which should be 'concern-driven scientific triggers'
  - ECHA, in close cooperation with member states and stakeholders should develop guidance on the application of the EOGRTS

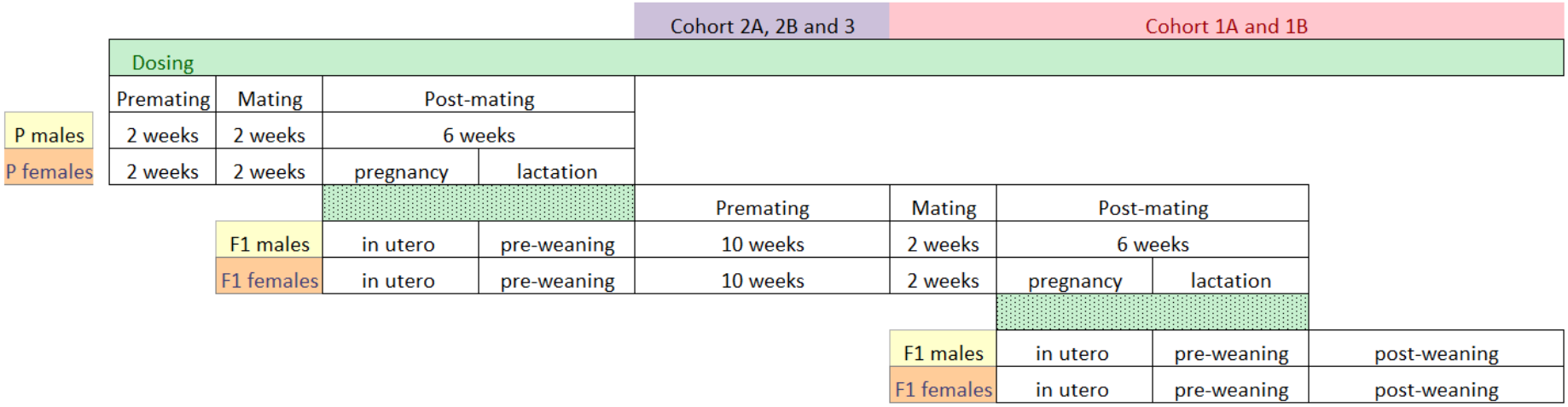
THE 2-GENERATION REPROTOXICITY STUDY (OECD 416)

	Dosing											
	Premating	Mating	Post-mating									
P males	10 weeks	2 weeks	6 weeks									
P females	10 weeks	2 weeks	pregnancy	lactation								
					Premating	Mating	Post-mating					
	F1 males	in utero	pre-weaning		10 weeks	2 weeks	6 weeks					
	F1 females	in utero	pre-weaning		10 weeks	2 weeks	pregnancy	lactation				
						F1 males	in utero	pre-weaning	post-weaning			
						F1 females	in utero	pre-weaning	post-weaning			

- Two generations of rats are produced from the starting parental animals: very animal intensive (~ 2600 rats)
- Retrospective analyses of existing data (mainly on biocides, agrochemicals and pharmaceuticals): in 3 out of 176 studies reprotox effects were observed in F2 and not in F1 offspring, but never severe enough to trigger classification

→ Strong societal pressure (NGO's, European parliament) to reduce animal use for substance testing

EOGRTS (OECD 443, WITH ALL 3 COHORTS)



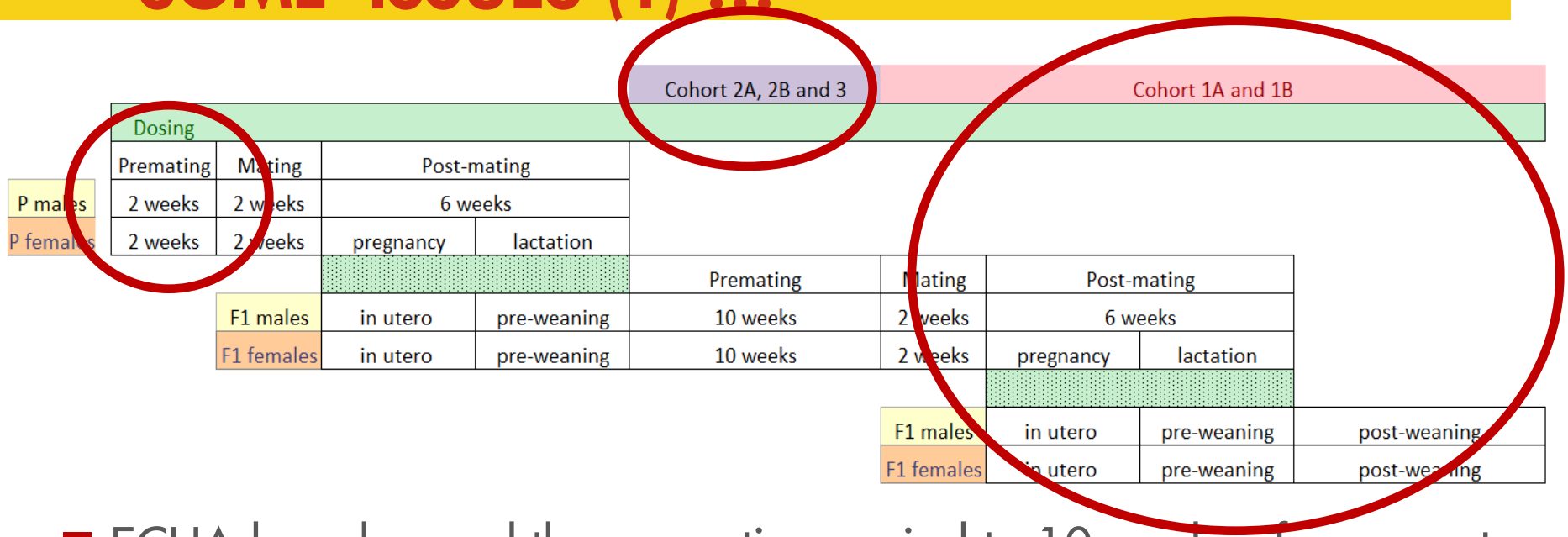
- The 'extensions' of the EOGRTS are the three cohorts: 1. Second generation, 2. Developmental neurotoxicity (DNT), and 3. Developmental immunotoxicity (DIT)
- A 'full-blown' EOGRTS, with all cohorts, is at least as animal intensive as the two-generation reproductive toxicity test
- For practical/organisational reasons, it should be known whether or not cohorts need to be included prior to commencing the study

EOGRTS (OECD 443, CORE STUDY)

	Dosing			
	Premating	Mating	Post-mating	
P males	2 weeks	2 weeks	6 weeks	
P females	2 weeks	2 weeks	pregnancy	lactation
				Post-weaning
F1 males			in utero	pre-weaning
F1 females			in utero	pre-weaning

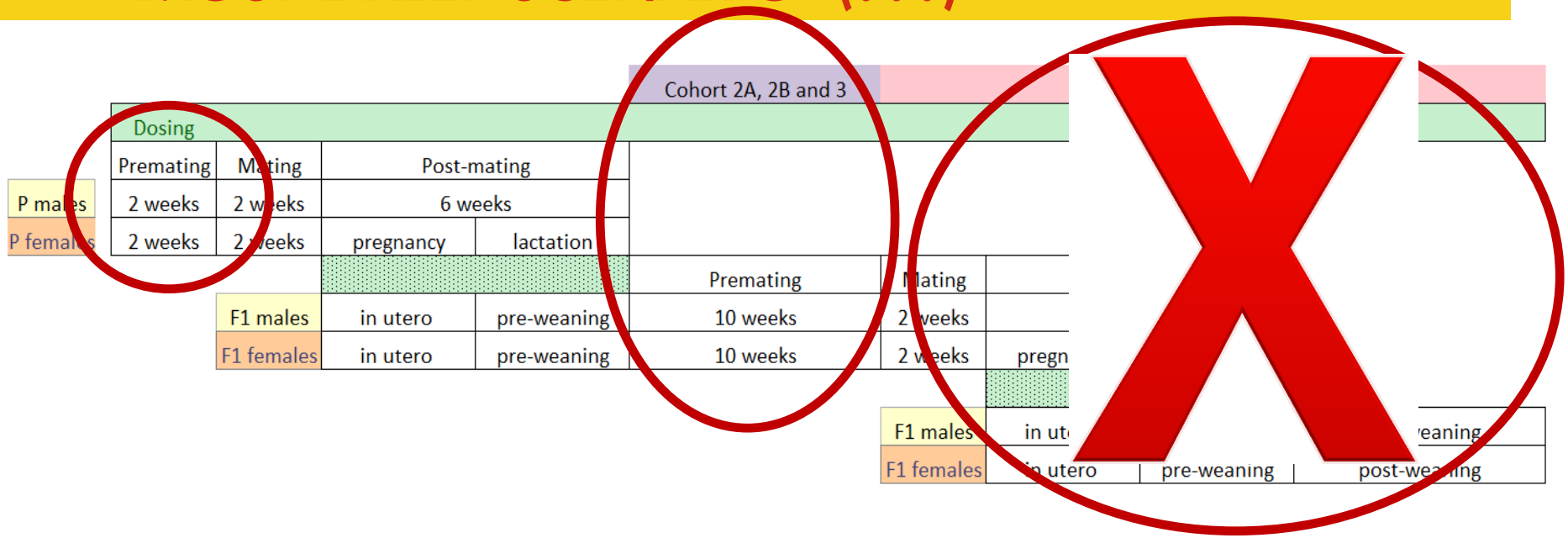
- The EOGRTS **without** the second generation gives a significant reduction in number of animals used (~1400 instead of ~2600), ***without a significant loss of information*** acquired from the study
- The DNT and DIT modules do ***not*** require more animals, but are considered to address endocrine disruptor issues
- The core study is expected to fulfil the basic regulatory requirements
- Big question: when will the additional cohorts be triggered ???

SOME ISSUES (1) …



- ECHA has changed the premating period to 10 weeks; if you want to deviate (i.e. follow OECD 443 !) you have to justify
- Triggers for conducting developmental neurotoxicity (DNT) and developmental immunotoxicity (DIT) have not been well defined
- Triggers resulting from observations during first part are highly impractical (and unknown at present)
- Triggers for second generation are also not well defined ('contra-indication' from existing data-base)

MOST LIKELY SCENARIO (???)



- Premating period will be extended to 10 weeks (OECD may adapt the guidance ??)
- One or both of the additional cohorts for developmental neurotoxicity (DNT) and developmental immunotoxicity (DIT) will become a 'standard requirement' based on Member State requests
- The second generation will ***not*** be required (issues with animal wellfare NGOs and Member States)

SOME ISSUES (2) …

- Not totally clear how ECHA will proceed with current Testing Proposals
  - Ask for new, revised proposals ?
  - Issue Decisions with/without consulting Registrants ?
- There is very limited experience with the EOGRTS:
  - limited historical control data will make interpretation difficult
- There is limited capacity with CRO's to conduct EOGRTS:
  - Prioritisation ?
  - Extend timelines ?
- Europe is not an island: what about regions with regulations that still require an OECD 416 ???

INTERPRETATION - INVERSE APPROACH (1)

'8.7.3. Extended One-Generation Reproductive Toxicity Study (B.56 of the Commission Regulation on test methods as specified in Article 13(3) or OECD 443), basic test design (cohorts 1A and 1B without extension to include a F2 generation), one species, most appropriate route of administration, having regard to the likely route of human exposure, if the available repeated dose toxicity studies (e.g. 28-day or 90-day studies, OECD 421 or 422 screening studies) indicate adverse effects on reproductive organs or tissues or reveal other concerns in relation with reproductive toxicity.

Could the legal text be interpreted as follows ?

- If the available information is *negative* there is *no need* for EORTS
- Can the EORTS be waived by generating screening studies ?

INTERPRETATION - INVERSE APPROACH (2)

8.7.3. An Extended One-Generation Reproductive Toxicity Study with the extension of cohort 1B to include the F2 generation shall be proposed by the registrant or may be required by the Agency in accordance with Article 40 or 41, if:

(a) the substance has uses leading to significant exposure of consumers or professionals, taking into account, inter alia, consumer exposure from articles, and

(b) any of the following conditions are met:

— the substance displays genotoxic effects in somatic cell mutagenicity tests *in vivo* which could lead to classifying it as Mutagen Category 2, or

— there are indications that the internal dose for the substance and/or any of its metabolites will reach a steady state in the test animals only after an extended exposure, or

— there are indications of one or more relevant modes of action related to endocrine disruption from available *in vivo* studies or non-animal approaches.

Could the legal text be interpreted as follows ?

- Cohort 1B will *not* be required if the following conditions are met:
- ADME studies indicate accumulation does not occur
- In vitro studies on MOA (AOP) show that the material is of no concern

INTERPRETATION - INVERSE APPROACH (3)_

An Extended One-Generation Reproductive Toxicity Study including cohorts 2A/2B (developmental neurotoxicity) and/or cohort 3 (developmental immunotoxicity) shall be proposed by the registrant or may be required by the Agency in accordance with Article 40 or 41, in case of particular concerns on (developmental) neurotoxicity or (developmental) immunotoxicity justified by any of the following:

— existing information on the substance itself derived from relevant available *in vivo* or non-animal approaches (e.g. abnormalities of the CNS, evidence of adverse effects on the nervous or immune system in studies on adult animals or animals exposed prenatally), or

— specific mechanisms/modes of action of the substance with an association to (developmental) neurotoxicity and/or (developmental) immunotoxicity (e.g. cholinesterase inhibition or relevant changes in thyroidal hormone levels associated to adverse effects), or

— existing information on effects caused by substances structurally analogous to the substance being studied, suggesting such effects or mechanisms/modes of action.

Other studies on developmental neurotoxicity and/or developmental immunotoxicity instead of cohorts 2A/2B (developmental neurotoxicity) and/or cohort 3 (developmental immunotoxicity) of the Extended One-Generation Reproductive Toxicity Study may be proposed by the registrant in order to clarify the concern on developmental toxicity.

Two-generation reproductive toxicity studies (B.35, OECD TG 416) that were initiated before 13 March 2015 shall be considered appropriate to address this standard information requirement.

The study shall be performed on one species. The need to perform a study at this tonnage level or the next on a second strain or a second species may be considered and a decision should be based on the outcome of the first test and all other relevant available data.'

Could the legal text be interpreted as follows ?

- DNT and DIT cohorts will *not* be required if the following are true:
- Evidence from *in vivo* or *in vitro* studies is negative
- MOA (AOP) shows no concern
- Structural analogy (read across) is possible to a non-active substance, *suggesting* no effect
- Other studies (MOA/AOP) can be done to demonstrate no-concern
- Does the Zebrafish count as 2nd species?

CONCLUSIONS

- The EOGRTS (OECD 443) is a done deal and meant to stay
- Existing and already started 2-generation reproductive toxicity studies (OECD 416) will remain/be valid for REACH
- Unclear what happens if an OECD 416 is required under another regulatory scheme
- Interpretation issues of the legal text with regard to the triggers (waivers ?) for the additional cohorts
- The Guidance, especially for triggers/waivers for the additional cohorts (1A, 1B, 2A, 2B and 3), needs to be more specific and with sound scientific underpinning
- There may be capacity problems with CRO's as well as issues with lack of historic control data which may hamper valid interpretation of results.