Alternative methods: what we have and what we need a regulatory view

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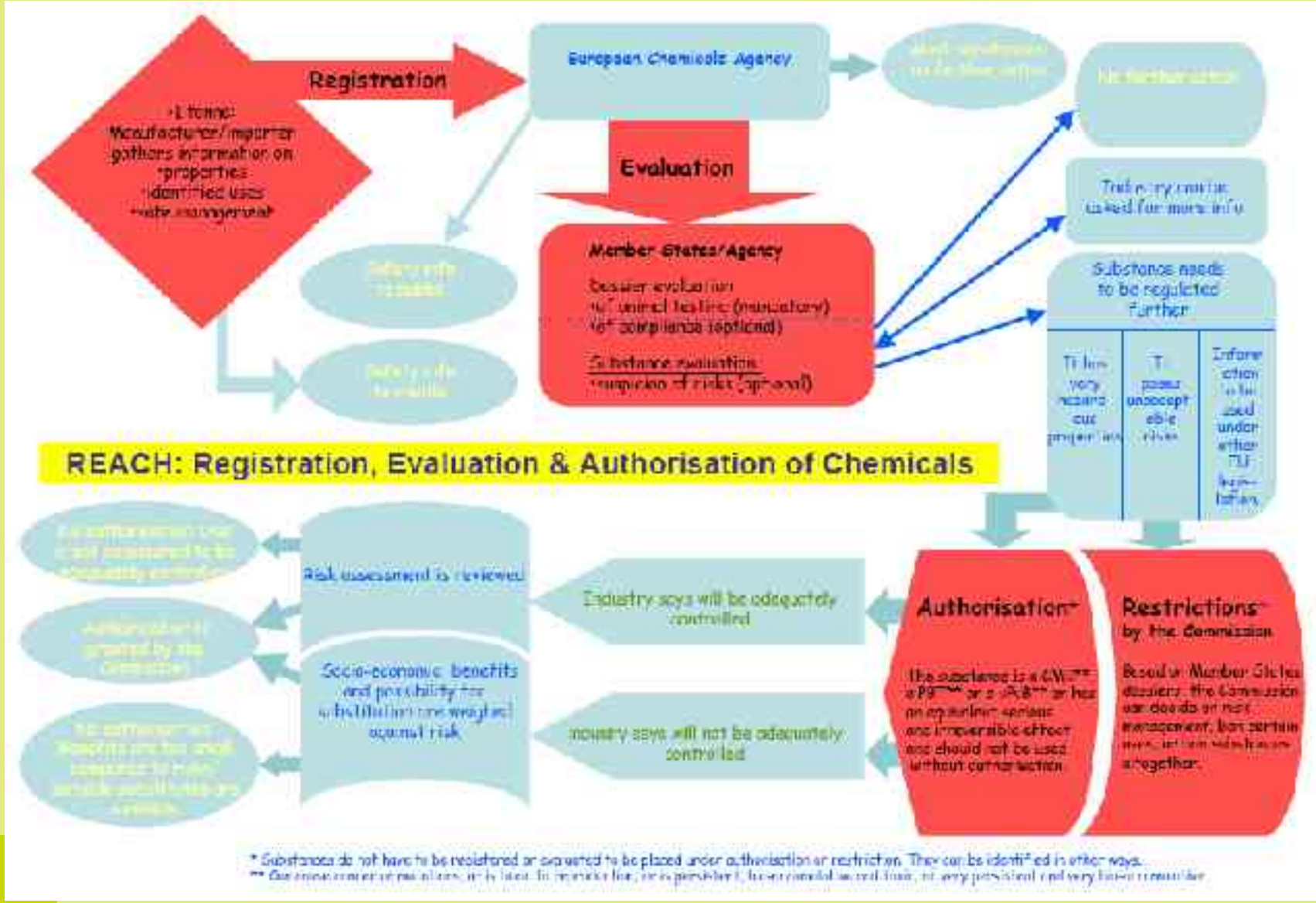
Aims of toxicity testing

- Risk assessment
  - On the basis of most sensitive parameter
  - Requires testing dedicated end points
- Classification and labelling
  - On the basis of specific toxicity
  - May arise from general tox testing or from dedicated toxicity testing
  - Requires testing dedicated end points

REACH


Registration
European Chemicals Agency
Evaluation
Member States/Agency
Substance needs to be regulated further
REACH: Registration, Evaluation & Authorisation of Chemicals
Risk assessment is reviewed
Industry says will be adequately controlled
Socio-economic benefits and possibility for substitution are weighed against risk
Industry says will not be adequately controlled
Authorisation*
Restrictions* by the Commission

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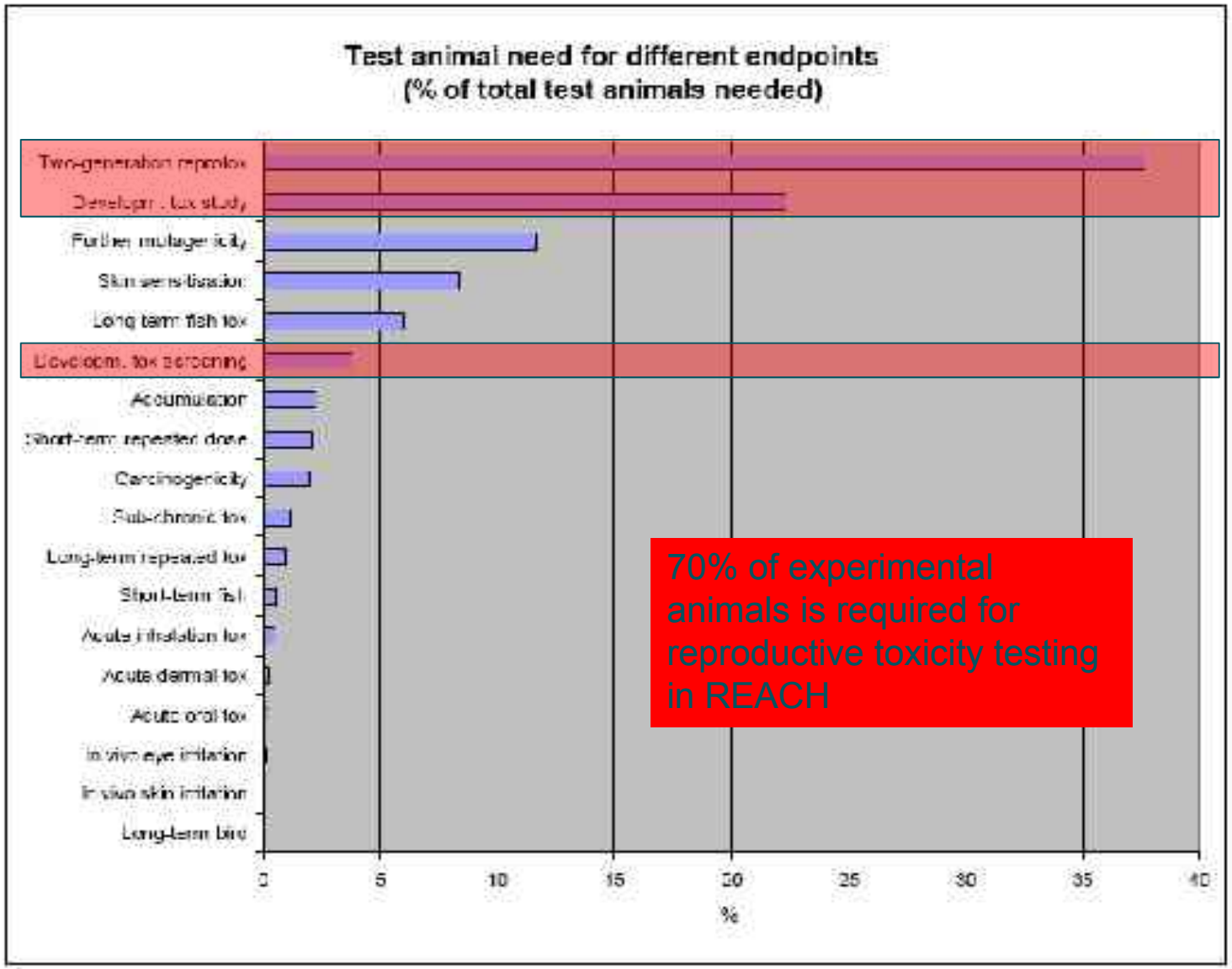

Van der Jagt et al., ECB 2004

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OECD GD 43

Figure 1 : Conceptual Framework for the Regulatory Hazard Assessment of Chemicals With respect to Mammalian Reproduction, based on increasing levels of information provided

Expected human & environmental exposure and use patterns
- hazard, *e.g.*, available toxicological data (enhanced TG 407)
- QSARs for blood / testis barrier
- QSARs for blood / placenta barrier
- QSARs for blood / breast milk barrier
- QSARs for blood / brain barrier

physical & chemical properties, *e.g.*, MW, reactivity, volatility, biodegradability

Level 1
Profiling and alerts based upon existing information

Level 2
In vitro **assays providing mechanistic data and focus on target aimed tests**

- ER, AR, TR receptor binding affinity
- transcriptional activation
- aromatase and steroidogenesis *in vitro*
- aryl hydrocarbon receptor recognition / binding
- embryonic stem cell tests
- *ex vivo* sperm test
- *ex vivo* oocyte test
- *in vitro* fertilization
- target cell toxicity
- Leydig cell viability
- fetal oocyte viability
- *in vitro* genetic toxicity

Level 3
In vivo **assays providing data about single mechanisms related to fertility/reproduction**

- Uterotrophic bioassay (estrogenic related)
- Hershberger bioassay
- non-receptor mediated hormone function
- copulation behavior

Level 4
In vivo **assays providing data about multiple mechanisms related to fertility/reproduction**

- male and female pubertal assays
- adult intact male assay
- reproductive screening test (TG 421)
- combined TG 421 / TG 407
- Segment I, II, III studies
- 1-generation assay (TG 415)
- 2-generation assay (TG 416)
- prenatal development (TG 414)
- developmental neurobehavior (TG 426)

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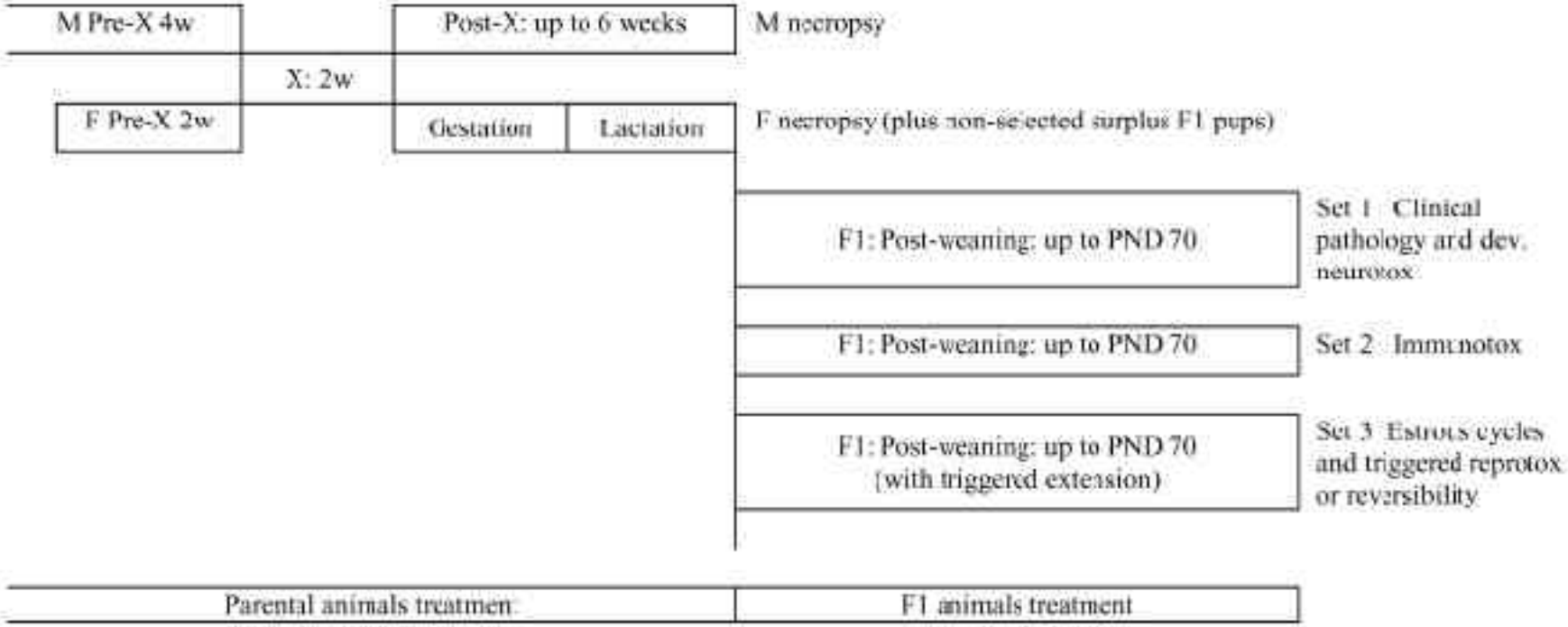
Novel in vivo test systems

- Reproductive toxicity screen (OECD421)
- OECD426 developmental neurotoxicity test
- OECD407 enhanced 28-day subschronic toxicity test
- Uterotrophic assay (OECD validation effort)
- Hershberger assay (OECD validation effort)
- Extended one-generation study (OECD task force)
- Juvenile exposure drug testing

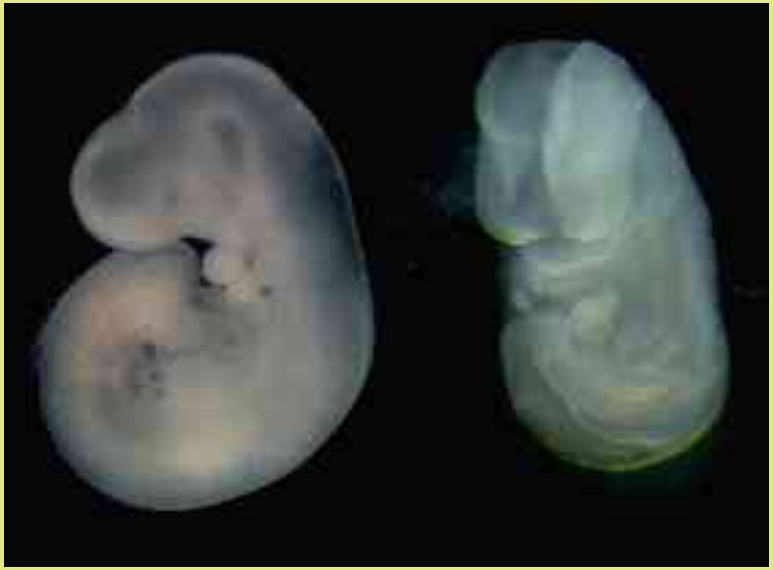
- OECD GD34 guidance: validation of new methods
- OECD GD43 guidance: reproductive toxicity testing strategy

Extended one-generation study

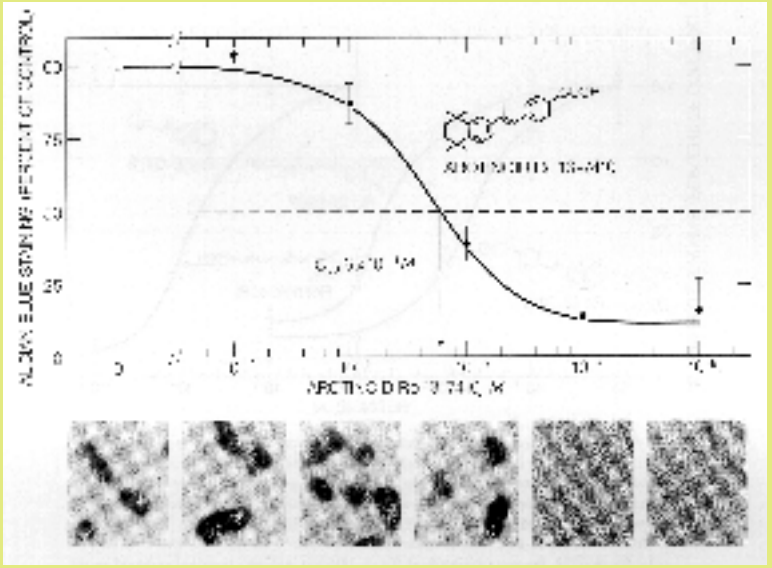


Cooper et al., 2006: Crit Rev Tox 36: 69-98.

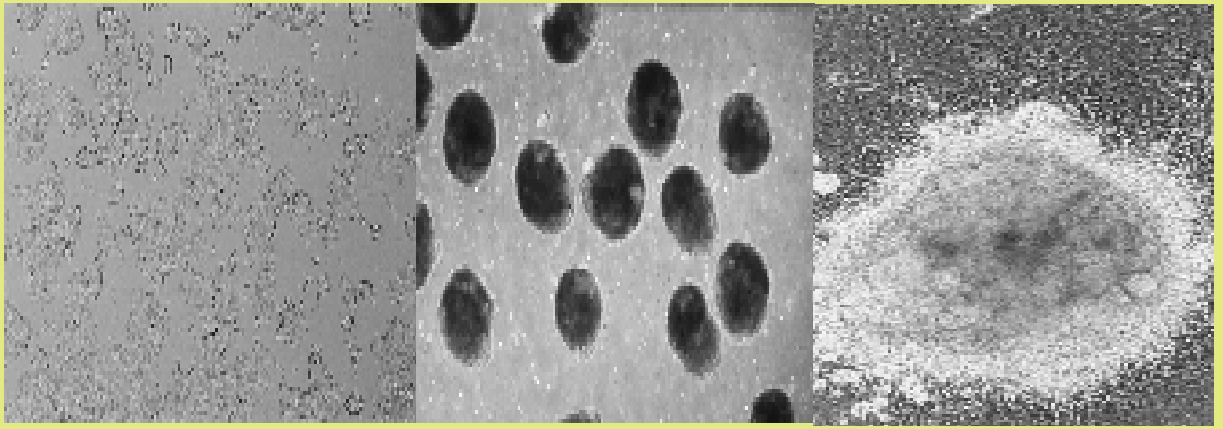
Developmental toxicity alternatives



WEC (photos Aart verhoef, RIVM)



MM (from Kistler, 1981)



EST (photos Dorien Verhallen, RIVM)

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Which are the new approaches that can help reduce animal testing resp. numbers used in toxicity testing?
What would be another area to address and target with new thinking, or new methodology?
What area can, or should be approached today, tomorrow? What's overdue ?

- Improve informative value of in vivo testing
  - relevant end points (add some to preclude extra tests)
  - remove superfluous end points (2nd generation in 2-gen study?)
  - benchmark dose-response design (replace NOEL)
  - integrated testing strategies (info-based waiving)
  - refrain from unnecessary screens (uterotrophic assay?)
- In vitro screens
  - applicability domain: end points and chemical classes
  - selected prescreening situation
- In silico
  - bioinformatics – systems biology
  - improve QSAR – read-across – category approach databases

Why only now that one addresses two-generation studies?

- The design was based on sound biological principles: only the second generation tests the effect of germ cell exposure on fertility
- REACH has shown that reproductive toxicity is the major animal using toxicology discipline
- Only now do we have the extensive database of 20+ years of experience allowing for a substantial retrospective data analysis
- Still, there may be compounds for which effects seen only in the 2nd generation have precluded marketing, and for which data have not been submitted to regulators. This will be a major point for discussion in decisionmaking on whether the 2nd generation can be omitted in general

Does this event have an exemplary character?

- It shows that we are at a crossroads in hazard assessment in general, where retrospective data analysis is feasible which may lead to innovation

- Care should be taken not to oversimplify, the basis should always remain a sufficient hazard information database

Do we need more realism in what can be achieved?

- Refinement of in vivo testing is likely to be more successful in reducing animal experimentation in the short run than introduction of alternatives
- In vitro alternatives need discussion about:
  - applicability domain
    - chemical classes
    - biological end points
  - their place in the testing strategy
- In vivo tests can be refined
  - additional end points in one animal as appropriate
  - possible reduction of generation study design
  - benchmark dose approach

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., submitted)

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thank you