# How to bring your registration dossier in compliance with REACH – Tips and Hints

Part 1

## Hints and Tips on Physico-chemical, environmental and human health related endpoints

27 September, 2012
15:00 - 17:00 Helsinki Time (GMT +3)

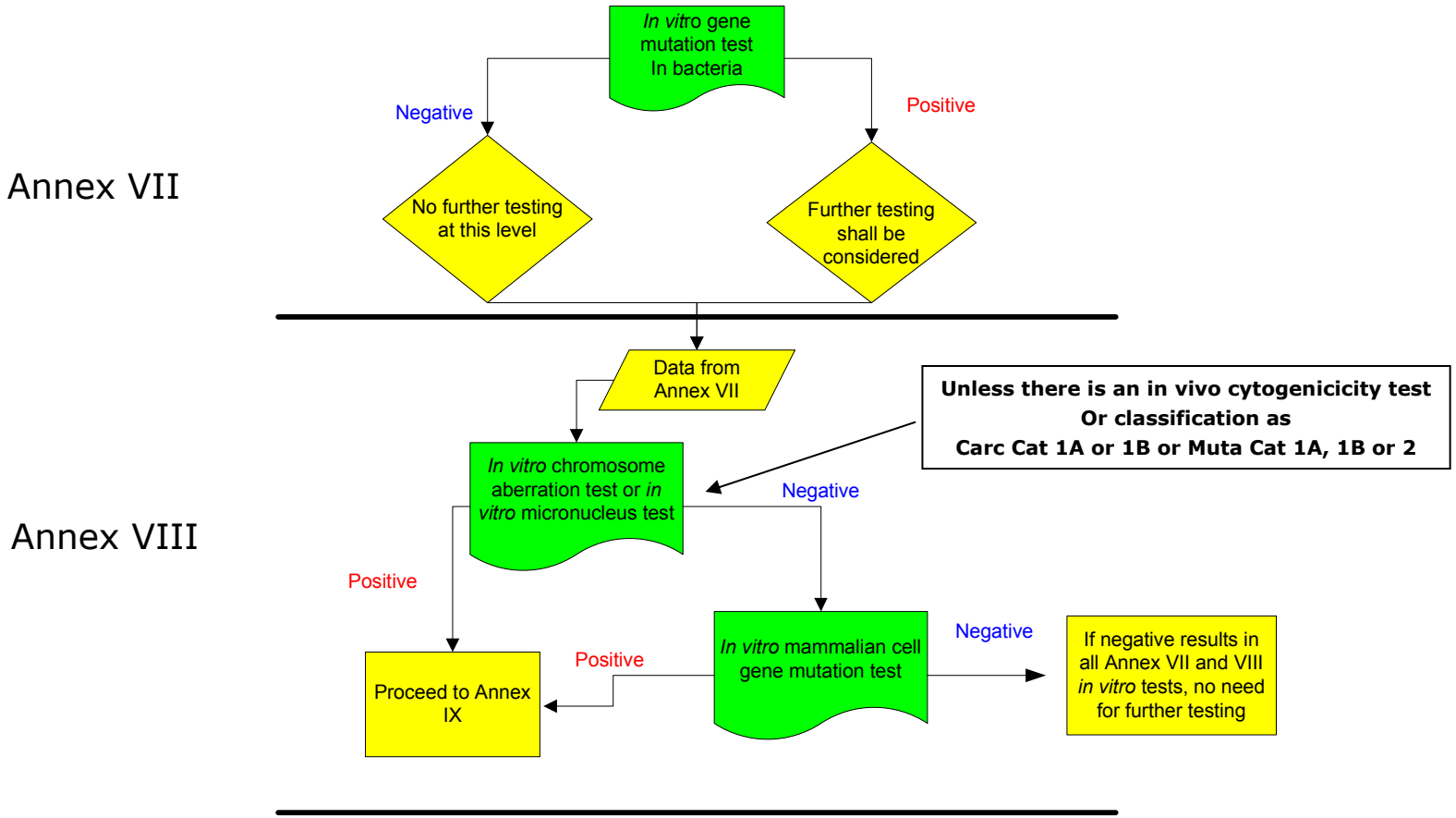
# Human Health endpoint

# Genotoxicity

**Speaker: Lars Nylund**

# Human Health endpoints
## Mutagenicity – information requirements

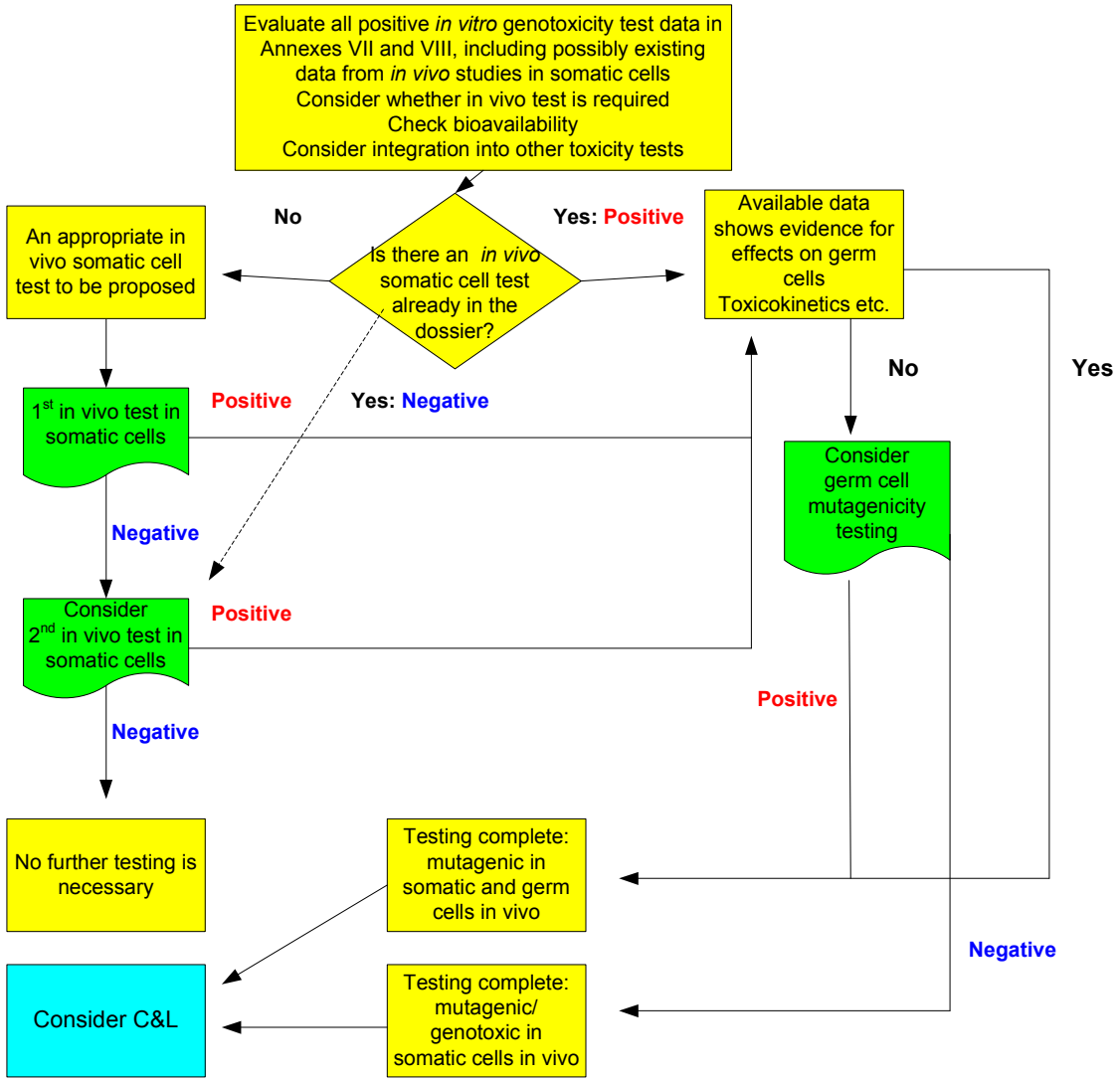
- Mutagenicity testing in REACH – a tiered approach



# Human Health endpoints

## Mutagenicity – information requirements

Annex IX and X



# Human Health endpoints
## Mutagenicity – In vitro testing: Reminders (1)

- Annex VII, 8.4.1. In vitro gene mutation study in bacteria
  - An acceptable bacterial gene mutation test should include ”fifth strain” (*Salmonella typhimurium* TA102 or *Escherichia coli* WP2 uvrA (pKM101))
    - According to the current OECD TG 471 (1997)/EU B.13/14
    - The mutagenic sensitivities of the combination of individual bacterial strains in the current TG is a key parameter in the meaning of Article 13(3) and Annex XI, section 1 provisions of REACH
    - Unless a submitted ”four strain” study is positive.
  - A bacterial gene mutation test using the Prival & Mitchel modification is recommended in addition to the standard test if the registered substance is an azo dye.

# Human Health endpoints
## Mutagenicity – In vitro testing: Reminders (2)

- Annex VIII, 8.4.2. In vitro cytogenicity study in mammalian cells
  - Current OECD TG 473 (1997) / EU B.10
    - **Key parameters: eg. Number of metaphase spreads analysed per dose (100 vs 200)**
    - **Unless a weight-of-evidence determination using expert judgement suggests that the data requirement may be fulfilled**

- NB: Examination of key parameters of studies conducted according to ”old” TG:s apply for all mutagenicity/ genotoxicity tests, including in vivo assays → see if the test is adequate in accordance with Article 13(3) and Annex XI, section 1

# Human Health endpoints
## Mutagenicity – In vivo testing: Reminders (1)

- Appropriate in vivo tests in somatic cells (Annex VIII, 8.4.)
  - Appropriate in vivo tests, depending on which types of in vitro tests were positive
    - Test types reflect **predominantly** **gene mutations** or **cytogenetic** effects (chomosome abberrations or aneugenic effects)
  - In vivo test methods, somatic cells – ECHA Guidance
    - Cytogenetic:
      - **In vivo mammalian bone marrow chromosome aberration test (CA in vivo)**
      - **In vivo mammalian erythrocyte micronucleus test (MN in vivo)**
    - Gene mutation:
      - **Unscheduled DNA synthesis (UDS) test with mammalian liver cells in vivo**
      - **Transgenic animal models (TGR)**
      - **[In vivo alkaline single-cell gel eletcrophoresis assay for DNA strand breaks (Comet assay)] – No OECD or EU test guideline / method**

# Human Health endpoints
## Mutagenicity – In vivo testing: Reminders (2)

- A second in vivo test in somatic cells shall be considered:
  - When there are positive results in both gene mutation and cytogenetic assays, in vitro
    - The second in vivo study shall be appropriate to cover the type of missing information (gene mutations or cytogenetic effects)

- In vivo gene mutation tests in somatic cells
  - Test Guidelines exist for the in vivo UDS test in liver cells and the TGR assay
    - Choice between these two test: UDS → genotoxicity test, target organ: liver; TGR → mutagenicity test, target organ/tissue: any tissue, site of contact
    - **ECHA seminar 4 October 2012: experts to discuss the two tests → recommendations on their use**

# Human Health endpoints
## Mutagenicity – In vivo testing: Reminders (3)

- Appropriate in vivo tests in somatic cells – other test types observed in registration dossiers
  - **The Dominant Lethal Assay (DLT)** – OECD TG 478/EU B.22
    - Designed to detect mutagenic effects (mainly cytogenetic events, such as gross chromosomal damage) in germ cells
    - Less sensitive than the in vivo CA and MN tests → not appropriate for covering (possible) cytogenetic effects in somatic cells if the result is negative
      - **If positive, apply weight-of-evidence determination using expert judgement to decide whether it concludes testing for mutagenic effects in somatic cells in vivo (cytogenetic effects) taking into account all the available data on mutagenicity/genotoxicity**
  - **The Comet assay, in vivo** – No OECD TG or EU Method
    - Mentioned in the ECHA Guidance
    - May be considered on a case-by-case basis by ECHA
      - **Depends on the test protocol used (degree of validation and extent of agreement among experts)**

# Human Health endpoints
# Mutagenicity – Conclusions

- **Mutagenicity testing is concluded when***:
  - Results from **adequate** in vitro studies are negative, or
  - Results from **adequate** and **appropriate** in vivo studies triggered by positive in vitro data are negative, or
  - Positive results from in vivo somatic cell studies are followed up by proposals for germ cell studies, or information about interaction of the registered substance with germ cells is provided, and
    - Classification is considered and the registration dossier is updated with a proposal for C&L

* Unless acceptable waivers are presented or weight-of-evidence determination using expert judgement is applicable

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# Thank you
# for your attention