Assessment of Reproductive Toxicity under REACH
July 2012

Walter Aulmann
Position Paper

“Toxic to reproduction“
Subcategories GHS / CLP

Developmental toxicity / teratogenicity

- Malformations of progeny, functional defects, reduced growth, damaged organs, functional impairment (immune functions, behaviour)

Impairment of fertility

- Reduced sperm quality, disruption of hormone status, impairment of libido, sexual behaviour
## Typology of tests

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## Developmental Toxicity

### OECD TG 414

**Species:**
- rats, rabbits

**Protocol**
- Treatment day 6 through 20 of gestation
- 3 dose groups, 1 control group
- Clinical observations during in-life phase
- Day 20 preparation of fetuses
- Visceral and skeletal analysis

**Biologically significant parameters**
- Maternal toxicity
- Embryo/ fetotoxicity
- Teratogenicity
Parameters for investigation

**Maternal toxicity**
- Body weight, water and food intake
- Clinical observations
- Necropsy

**Embryo- Fetotoxicity**
- Uterus- and plazenta weights
- Number of corpora lutea
- resorptions
- Number and weight of fetus
- Sex ratios / life lethal birth

**Teratogenity**
- Skeletal changes
- Visceral changes

Focus: potential harm to unborn child
Impairment of male / female fertility

**OECD TG 415 / OECD 416**

**Species:**
- Rats, mice

**Protocol**
- Treatment (7d/week)
  - males: over a full spermatogenesis-cycle
  - females: over 2 estrus-cycles und and during gestation and breeding
- Clinical observation
- Necropsy and histopathology

**Biologically significant effect descriptors**
- Reproductive integrity and performance (P / F1 (F2))
Reproductive integrity and performance

Parameters for investigation in the OECD 415/416

- Gonads
- Estrous cycle
- Mating behaviour
- Conception
- Birth
- Lactation
OECD 422 screening test *

Species:
- Rats

Protocol
- Treatment (7d/week)
  - Males: 4 w
  - Females: approx. 7 w
  - F1: 4 d
- Clinical observation
- Necropsy and histopathology

Biologically significant effect descriptors
- Repeated dose toxicity
- Reproductive performance

* OECD 421: similar approach but not combined with repeated dose toxicity

For differences see back-up slide
Historical background

**Trigger**

- Data gaps for many High-Production-Volume (HPV)- substances in terms of reprotox

**Aim**

- Identification of critical substances
- High through-put solution

**What they are not aiming at**

- Substitution of definitive studies (OECD 414/415/416)
OECD-Screening tests 421 / 422

A rough comparison

OECD 421/422

Investigations related to reprotox ± the same

OECD 422

+ Hematology, clinical chemistry, urinalysis
+ all organs: histopathology, organ weight

→ Information about repeated dose toxicity → 'combined test'

Conclusion: OECD 422 is given preference over OECD 421
OECD test 407

OECD 407 (28-day-study) is the conventional tier-1-method in Europe to investigate repeated dose toxicity of industrial chemicals.

It was upgraded in 1995 to cover also reproductive parameters.

With respect to fertility the prediction value of the OECD 407 28 days study is comparable to the OECD 421/422.
Detection of detrimental effects on fertility
„Histology of male reproductive organs is more sensitive than conventional fertility parameters“ ([Ulbrich und Palmer, 1995, Reuter et al, BUA, Mangelsdorff et al])
„Detailed histology is applicable also to investigate female fertility“ (Sanbuisho, et al.)

Detection of developmental toxicity
„insufficient“ [Reuter et al, 2003, BUA]
Animal numbers compared

OECD 414, 415, 416:
- 20 M, F

OECD 422:
- 10 M, F

OECD 407:
- 10 M, F

Reduced statistical resolution
- 1/10-Problem

Question of relevance of non significant effects
Investigation of Reproductive Toxicity

REACH requirements for 10 – 100 ton (annex VIII):
Screening tests (OECD 421 or OECD 422)

- Pros:
  - Quick approach
  - Lower number of animals compared to definitive studies
- Cons:
  - Low statistical resolution
  - No investigation of developmental effects (terata, malformations)

Also includes some insights on repeated dose toxicity (Combined test)

However:
Screening tests are mandatory under REACH!
No freedom of choice for higher tier (definitive) tests!
Higher tier testing requires prior time-consuming ECHA approval
### REACH annex VIII data requirements

Current legislation (1) – mandatory without alternative

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# REACH annex VIII data requirements

As an alternate approach (GT-AK-RegTox proposal)

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„Detailed histology is applicable also to investigate female fertility“ (Sanbuisho, et al.)

Detection of developmental toxicity
Literature

BUA-Bericht 229
(Februar 2001)

Evaluierung der OECD-Screening-Tests 421
(Reproduction/Developmental Toxicity Screening Test) und 422
(Combined Repeated Dose Toxicity Study with the Reproduction/
Developmental Toxicity Screening Test)

S. Hirzel
Wissenschaftliche Verlagsgesellschaft
Evaluation of OECD screening tests
421 (reproduction/developmental toxicity screening test) and
422 (combined repeated dose toxicity study with the
reproduction/developmental toxicity screening test)

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Beate Holzum, d and Frank Welsch e

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b Federal Institute for Risk Assessment, Berlin, Germany
c BASF-AG, Ludwigshafen, Germany
d BAYER AG, Wuppertal, Germany
e Orbitox, Chapel Hill, NC, USA

Received 24 April 2002
Proposal 2012 *

[...]

Registrants should be allowed to select between these two options, either the existing approach (OECD TG 421/407 and alternatively TG 422) or the approach proposed in this paper (OECD TG 407 plus TG 414).

* „German Society of Toxicology, Committee for Regulatory Toxicology“
Questions ?
Reproduction Cycle

What we are talking about
Illustration on skeletal investigations

Rat fetus

kindly provided by J. Buschmann, ITEM, Fraunhofer, Hannover)
OECD tests 414 / 415 / 421/422

A comparison of study designs

OECD 415

pre-mating

10 w

OECD 421/422

mating

3/2 w

OECD 414

pre-mating

10 w

F

2 w

3/2 w

F1

3 w

4 d

Lactation

gestation

3 w

gd6

birth

3 w

4 d

OECD 414

pre-mating

10 w

M

10 w

3/2 w

OECD 415

pre-mating

10 w

M

10 w

3/2 w

OECD 421/422

mating

3/2 w
Guidance on Evaluation of Reproductive Toxicity Data

Monograph No. 31