Ahtiainen Jukka | 15.02.2011

# *OECD:n kemikaalitestimenetelmät ja EU:n testimenetelmäasetus*

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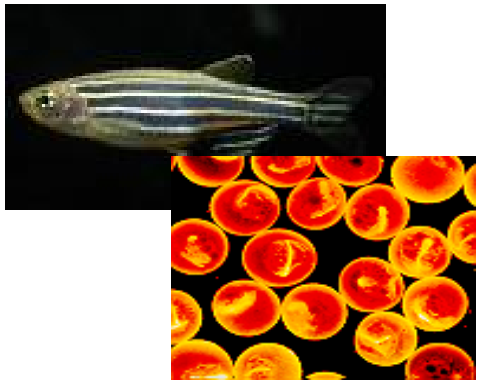
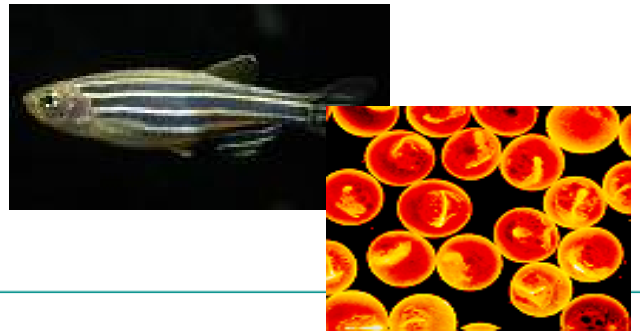
## *Sisältö*

- Johdanto - miksi OECD:n testiohjeet?
- OECD:n testimenetelmät
- EU:n testiohjeasetus
- Pohjoismainen yhteistyö- Nord-UTTE
- Ajankohtaista viimeiset vuodet ja nyt
  - Hormonaalisten vaikutusten testaus esimerkkinä



# *OECD:n testimenetelmät ja EU:n testiohjeasetus*

- OECD:n tausta taloudellisessa yhteistyössä ja kehityksessä
- OECD:n menetelmät ja MAD
- OECD:ssä ei ole rakennettu testausstrategioita, vain työkalupakki eri vaikutusten havaitsemiseksi
- Hyväksytyt EU-lainsäädännön tarvitsemat OECD- menetelmät tuodaan EU:n testiohjeasetukseen (EY 440/2008)



# *Reasons for harmonization*

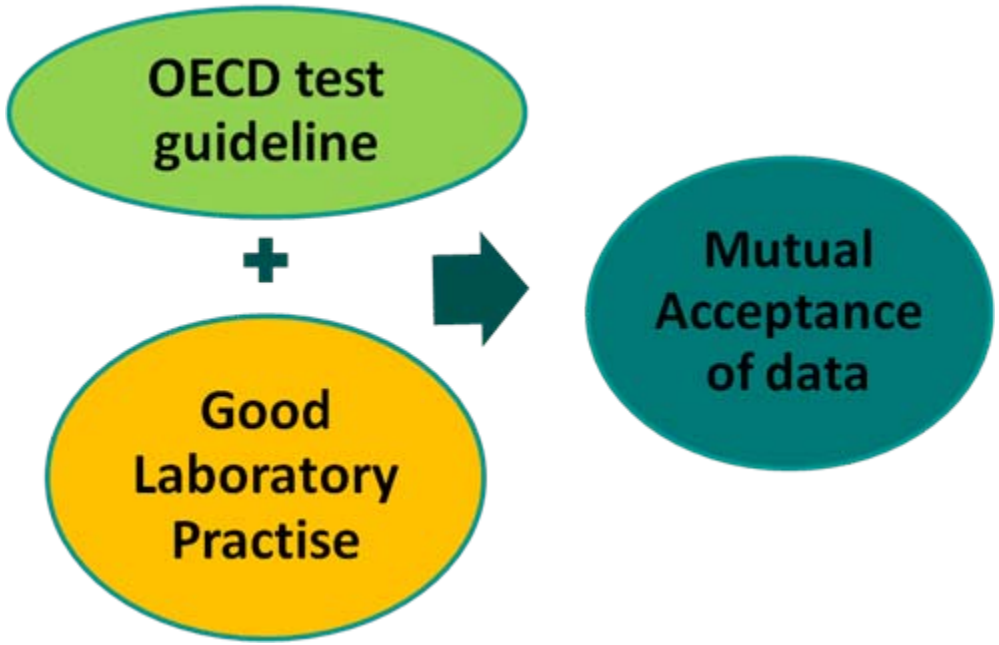




- **OECD COUNCIL DECISION ON MUTUAL ACCEPTANCE OF DATA IN AN ASSESSMENT OF CHEMICALS INCLUDING PESTICIDES**
- ***"... THE DATA GENERATED IN THE TESTING OF CHEMICALS IN OECD MEMBER COUNTRY IN ACCORDANCE WITH OECD TGS AND PRINCIPLES OF GLP SHALL BE ACCEPTED IN OTHER MEMBER COUNTRIES FOR PURPOSES OF ASSESSMENT AND OTHER USES RELATING TO THE PROTECTION OF MAN AND THE ENVIRONMENT."***
- **MAD IS NOT THE HARMONIZATION OF REQUIREMENTS FOR TESTING**

MAD = Mutual Acceptance of data

# *Why to have OECD methods?*




- *Binding OECD countries and selected non-members*
- *Avoids duplication of testing*
- *Reduces use of animals*
- *160 million euros saved each year (2010)*
- *"easily" adopted to EU regulation for EU needs*

# *OECD Test Guidelines and Guidance Documents*

## TEST GUIDELINE

- Regulatory need
- Covered by MAD
- Fixed test protocol with validity criteria
- Thorough validation needed with fixed protocol

- Takes time and resources and cumbersome to update

## GUIDANCE DOCUMENT

- Regulatory need
- **Not** covered by MAD
- Can be a test method, or it provides technical guidance for the use of test guideline
- Validation could be limited

- Faster to develop and revise

# *Coverage and objectives of the OECD TGs*

**ORIGINAL PUBLICATION 1981: 51 TEST GUIDELINES**

**LATEST AMENDMENT 2010 > 130 NEW OR UPDATED TGS**

**AVAILABLE FREE OF CHARGE SINCE JANUARY 2007**

**(WWW.SOURCEOECD.ORG)**

**GUIDANCE DOCUMENTS, REPORTS, DETAILED REVIEW PAPERS (NOT COVERED BY MAD) (WWW.OECD.ORG/ENV/TESTGUIDELINES)**

# *Coverage and objectives of the OECD TGs*



**NEW AND UPDATED TGS ARE DEVELOPED TO:**

- Meet the regulatory needs of member countries
- Have broad applicability (industrial chemicals, pesticides, biocides and others)
- Address animal welfare aspects (3Rs)
- Reflect scientific progress
- Improve cost-effectiveness of the assessment

3Rs = Reduce, Refine and Replace

# *Coverage and objectives of the OECD TGs*





**SECTIONS AND COVERAGE**

- Section 1: Physical chemical properties (22)
- Section 2: Effects on biotic systems (24)
- Section 3: Degradation and accumulation (14)
- Section 4: Health effects (51)
- Section 5: Other TGs (pesticide residues, efficacy, some in vitro methods, specific nanomaterial effects ?)

*Applicability of OECD test guidelines for regulatory testing of nanomaterials*

# *OECD TG development and validation*

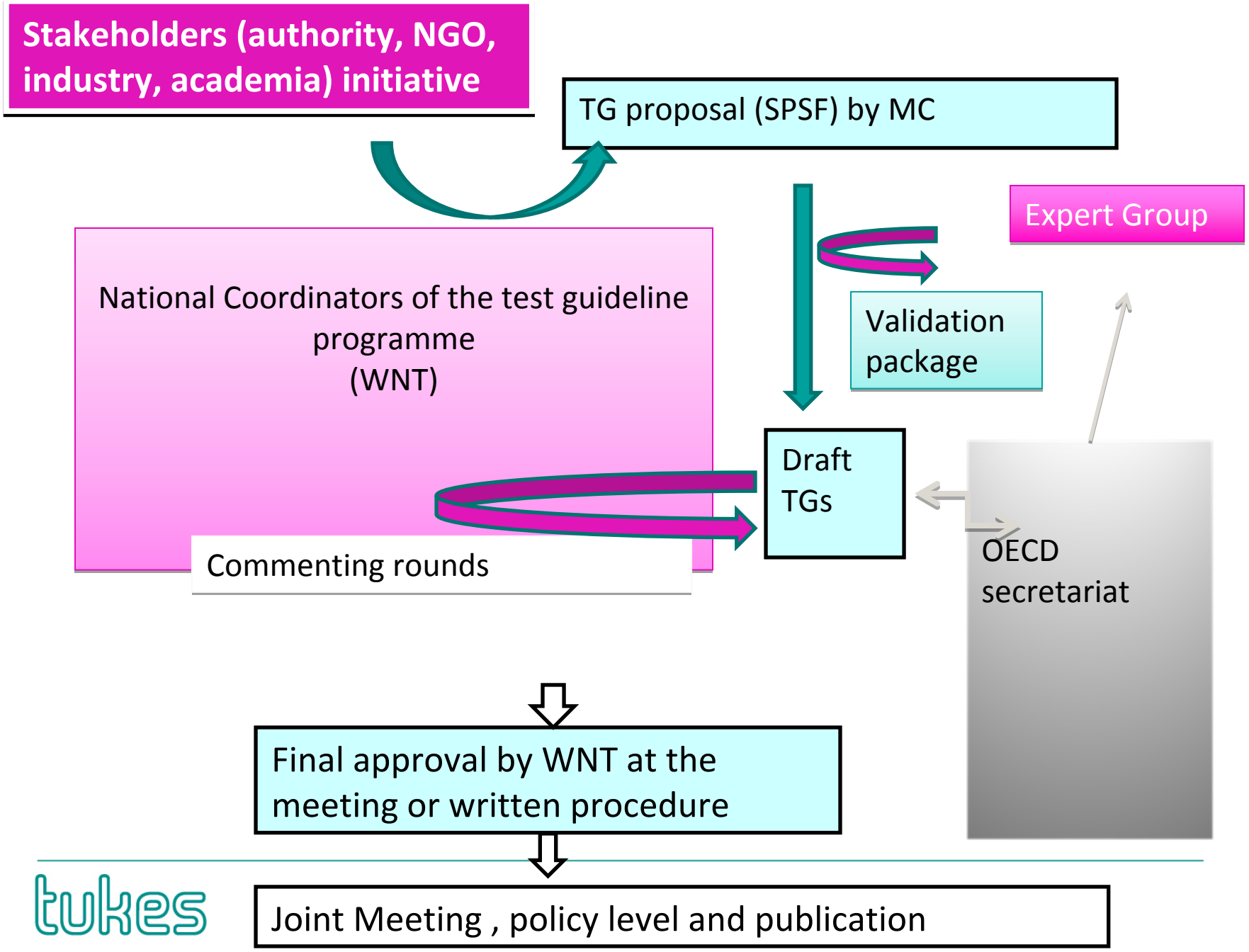
**TEST GUIDELINE PROGRAMME IS MANAGED BY NATIONAL COORDINATORS FROM MEMBER COUNTRIES (MC) AND EC INVOLVING ALSO:**

- Industry experts and other NGOs
- Observing countries

**DECISIONS ALWAYS BY CONSENSUS BETWEEN COUNTRIES**

**PROPOSAL FOR NEW WORK PROPOSED BY STANDARD PROJECT SUBMISSION FORM (SPSF) BY A MEMBER COUNTRY (OR EC)**

**TG IS ENDORSED BY NATIONAL COORDINATORS AFTER CONSULTING OF NATIONAL AND OTHER EXPERTS (CIRCULATIONS, MEETINGS)**


Stakeholders (authority, NGO, industry, academia) initiative
TG proposal (SPSF) by MC
Expert Group
Validation package
National Coordinators of the test guideline programme (WNT)
Draft TGs
Commenting rounds
OECD secretariat
Final approval by WNT at the meeting or written procedure
Joint Meeting , policy level and publication
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Ahlainen 2010/Nanotoxicology
2/17/2011

# *OECD TG development and validation*





**VALIDATION OF A TEST METHOD FOR REGULATORY USE:**

- Clear ”endpoint” with biological/ecological relevance
- Regulatory need and robustness to satisfy this
- Technical validation = reproducibility, repeatability, sensitivity

- OECD Guidance Document 34 (2005)- Principles and guidance for validation

# *OECD TG development and validation – Guidance Document 34 (2005)*

**DEFINITION OF A TEST METHOD**

**APPROACHES TO VALIDATION:**

- Prospective validation
- Retrospective validation and modular approach

**DESIGN AND CONDUCT OF VALIDATION STUDIES**

**INDEPENDENT EVALUATION- PEER REVIEW**

**REGULATORY ACCEPTANCE OF VALIDATED TEST**

**SUPPORTING DOCUMENTATION FOR NEW TESTS**

**EXAMPLES OF VALIDATION**

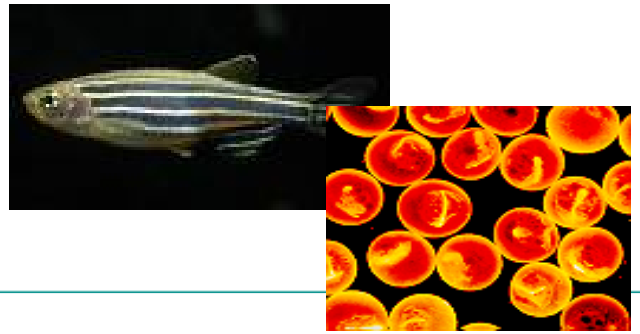
# OECD:n menetelmät hormonihäirikköjen testaamiseksi

- Onko erityistä huolta hormonihäiriköistä?
- Mikä merkitys, jos sellainen kemikaali olisi - eikö lisääntymismyrkyllisyys riitä?
- Vaikutus testiohjeiden kehitykseen, niin toksikologian ja ekotoksikologian puolella.
- ”kymmenen vuotta ja huono testiohje”

*OECD:n endokriinitestit ja ohjeistus*

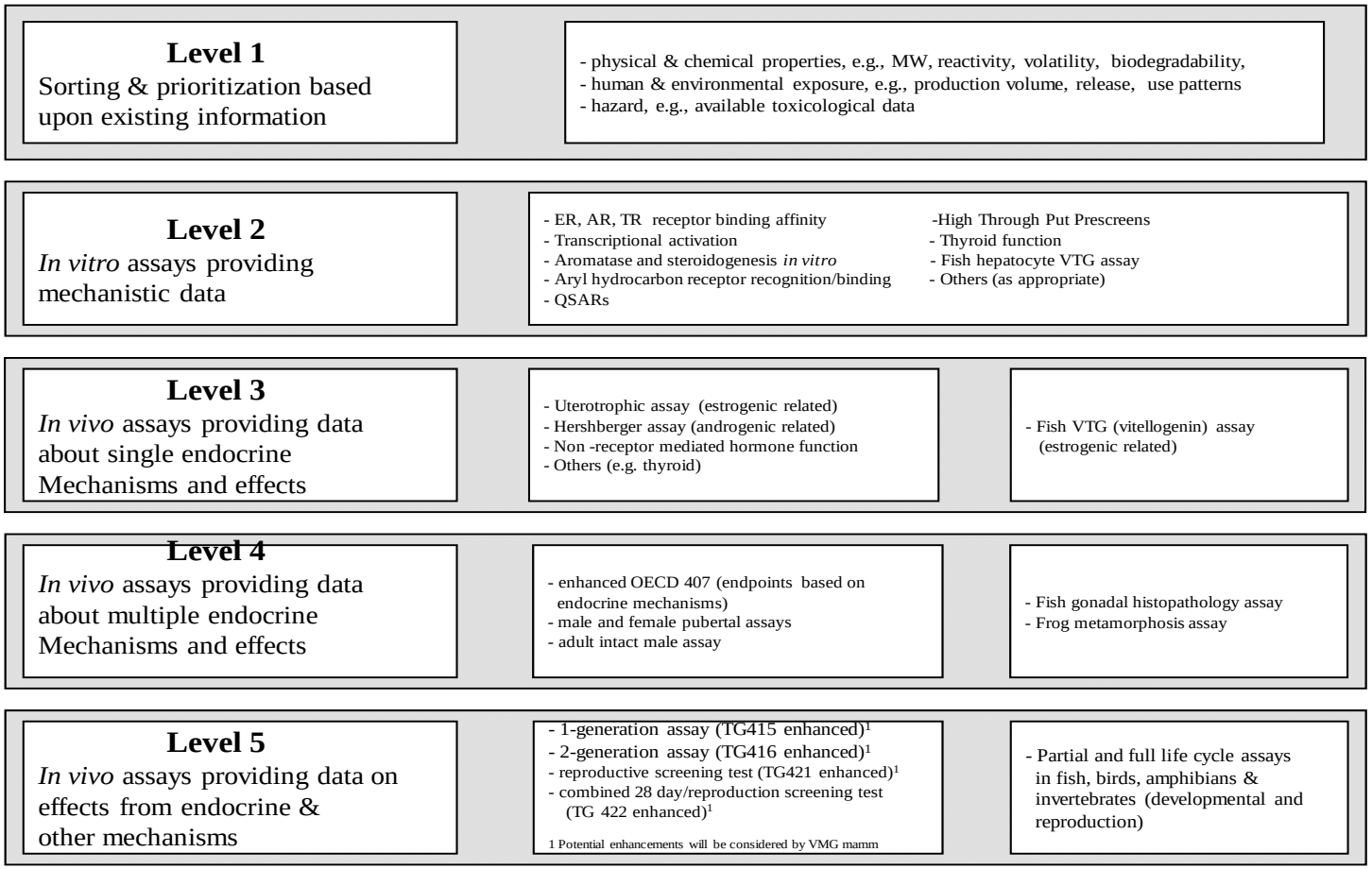
# *Conceptual framework for ED assessment*

- *Endocrine modalities generally covered (vertebrates):*
  - *Estrogen receptor mediated*
  - *Androgen receptor mediated*
  - *Thyroid hormone mediated*
  - *Steroidogenesis interference (new)*
- *Invertebrate hormonal system is not covered e.g. juvenile hormones…*



*OECD:n endokriinitestit ja ohjeistus*

# *Conceptual framework for ED assessment 2001*

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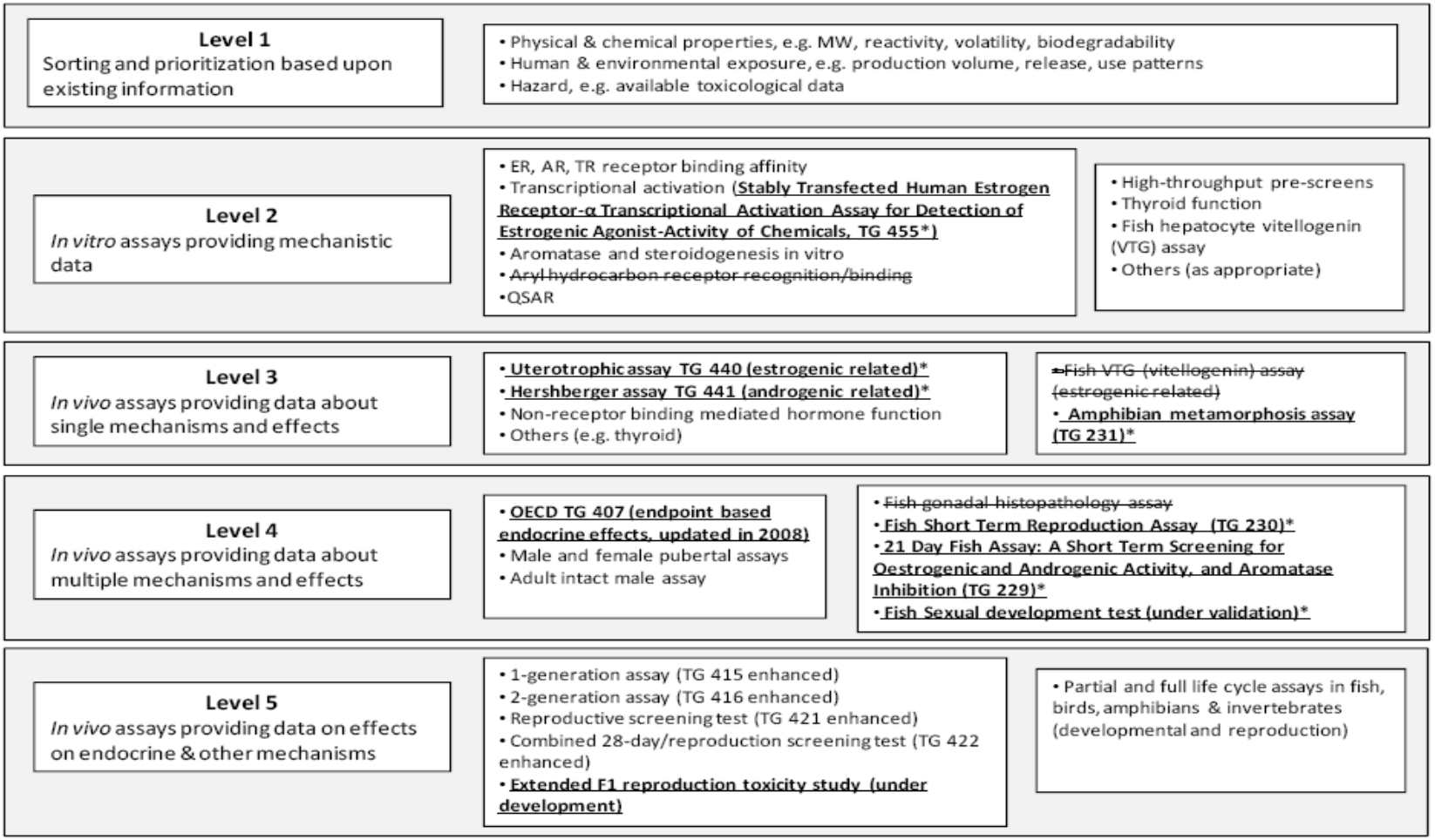
*OECD:n endokriinitestit ja ohjeistus*

# *Conceptual framework for ED assessment 2010*




* TGs approved by the WNT **Assays in bold – new added TGs** ~~Assays strikethrough~~ – deleted assays

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*OECD:n endokriinitestit ja ohjeistus*

## *Conceptual framework for ED assessment 2010 -ecotoxicology*

| | Tools in the box | | |
|---|---|---|---|
| **Level 1**<br>Sort and prioritize | • Phys-chem properties, degradation<br>• Environmental exposure (exposure scenarios)<br>• Available hazard data (tox or ecotox) | | |
| **Level 2**<br>*In vitro* testing for mechanistic data | • ER, AR, TR receptor binding, transcriptional activation, steroidogenesis, aromatase<br>• Hepatocyte VTG induction | | • QSARs<br>• Read across<br>• Grouping |
| **Level 3**<br>*In vivo* testing for single effects | | | |
| **Level 4**<br>*In vivo* testing for multiple effects | • Fish short term repro TG 229<br>• Fish 21-day screen TG 230<br>• Fish sexual development test (FSDT) | | |
| **Level 5**<br>*In vivo* testing for integrated effects | • Bird reproduction test TG 206<br>• Fish full lifecycle tests | | • Invertebrate reproduction and development tests (Daphnia, Chironomids TG 233, Collembola, Mysids, Molluscs) |

*OECD:n endokriinitestit ja ohjeistus*

# *In vitro- screenausmenetelmät*

- **OECD TG 455**: The stably transfected human ER transcriptional activation assay (ER STTA), agonist + antagonist guidance

Not yet adopted by the OECD:

- ER binding assay (US EPA)
- AR binding assay (US EPA)
- H295R steroidogenesis assay (draft OECD TG, US EPA)
- Aromatase assay (US EPA)

*OECD:n endokriinitestit ja ohjeistus*

# *Nisäkästestit*

*Screening tests:*

- **OECD TG 440**: Uterotrophic bioassay for E in rodents (including GD for anti-estrogenicity)
- **OECD TG 441**: Hershberger bioassay for A and anti-A in rats (including GD for weanling test)
- **OECD TG 407**: Repeated dose 28 day oral toxicity study in rodents

*Other test with endocrine modulated endpoints:*

- **OECD TG 415**: one-generation reproduction toxicity study
- **OECD TG 416**: two-generation reproduction toxicity study (including)
- Also other long term test: **OECD 408** (90d), **OECD 421, 422, OECD 452, 453**

*To be adopted by OECD:*

- Extended one-generation reproductive toxicity study

## *Uterotrophic bioassay in rodents OECD TG 440*

- **Estrogens (♀ uterine wet weight and dry weight ↑);**
- **Anti-estrogens (♀ stimulated uterine weight ↓);**
- **(Optional others *e.g.* histopathologic changes in uterus/vagina).**

## *Hershberger bioassay in male rats TG 441*

- **Androgens (♂ weights of ventral prostate, seminal vesicles, LABC, cowpers glands, glans penis ↑);**
- **Anti-androgens (weights of testosterone stimulated ventral prostate, seminal vesicles, LABC, cowpers glands, glans penis ↓);**
- **Optional others *e.g.* testis weight, changes in serum hormones.**

**Note: weanling H assay does not include glans penis.**

*OECD:n endokriinitestit ja ohjeistus*

# *Muut selkärankaistestit*

*Screening tests:*

- **OECD TG 229**: Fish short term reproduction assay (FSTRA)
- **OECD TG 230**: 21 day fish assay and androgenised female stickleback screen (AFSS)
- **OECD TG 231**: Amphibian metamorphosis assay (AMA)

*Other tests which could include endocrine modulated endpoints:*

- **OECD TG 206**: avian reproduction test

*To be adopted by OECD:*

- Fish sexual development test (FSDT)
- Fish lifecycle toxicity test (FLCTT)

*OECD:n endokriinitestit ja ohjeistus -kalatestit*

# *Fish 21-day assay TG 230*

- **Estrogens (♂VTG ↑);**
- **Anti-estrogens (♀VTG↓);**
- **Androgens (♂ 2o sex characters in ♀);**
- **Aromatisable androgens (♂VTG ↑);**
- **Aromatase inhibitors (♀VTG↓).**

**Note that this assay does not reliably detect anti-androgenic activity.**

- **Androgenized female stickleback assay (AFSSA)**

*OECD:n endokriinitestit ja ohjeistus- kalatestit*

# *Fish sexual development test (FSDT) draft*

- **Estrogens (♂VTG ↑; phenotypic sex ratio ♀↑);**
- **Androgens (phenotypic sex ratio ♂↑; ♀ spiggin ↑ in stickleback);**
- **Aromatase inhibitors (♀VTG↓; phenotypic sex ratio ♂↑);**
- **Anti-estrogens (♀VTG↓; sex ratio ♂↑;);**
- **Anti-androgens (♂ spiggin ↓ in stickleback; sex ratio ♀↑)**
- **Optional endpoints – gonadal histopathology; genetic sex in stickleback and medaka.**

*OECD:n endokriinitestit ja ohjeistus*

# *OECD:n ohjeisto (GD) testitulosten tulkinnasta*

Sille on sovittu seuraavat kolme tavoitetta:

- ***"Support regulatory authorities 'decision on the potential human health and ecological hazards of chemicals <u>when they receive test results</u> from a (draft) TG related to EDC screening/testing;***
- ***How to interpret the outcome of individual tests and how to increase evidence on whether or not a substance may be an EDC. Testing strategies or guidance on interpretation from a suite of tests are not given.***
- ***To minimise animal testing globally through a two step process"***

*OECD:n endokriinitestit ja ohjeistus*

# *GD on EDC assessment- Fish Sexual Development Test- interpretation of results*

| FSDT-test | *In vitro* (MOA) | Other *In vivo* | WoE | Next steps | Other |
|---|---|---|---|---|---|
| **+** | **+** | **+** | Hormonal/apical ?<br>- adverse? | Regulatory decision (?) | -test design for RA?<br>-FFLCT/MMLC |
| **+** | **+** | **-** | Hormonal/apical ?<br>- adverse? | Regulatory decision (?) | -test design for RA?<br>-FFLCT/MMLC |
| **+** | **+** | **0/Eq** | Hormonal/apical ?<br>- adverse? | Regulatory decision (?) | -test design for RA?<br>-FFLCT/MMLC |
| **+** | **-** | **+** | -effects-yes<br>-hormonal/advers? | Regulatory decision (?)<br>- Other MOA? | -test design for RA?<br>-FFLCT/MMLC |
| **+** | **-** | **-** | -effects-yes<br>-hormonal/advers? | Regulatory decision (?)<br>- Other MOA? | -test design for RA?<br>-FFLCT/MMLC |
| **+** | **-** | **0/Eq** | -effects-yes<br>-hormonal/advers? | Regulatory decision (?)<br>- Other MOA? | -test design for RA?<br>-FFLCT/MMLC |
| **+** | **0/Eq** | **+** | -effects-yes<br>-hormonal/advers? | Regulatory decision (?)<br>- Other MOA? | -test design for RA?<br>-FFLCT/MMLC |

# *GD on EDC assessment- Fish Sexual Development Test- interpretation of results*

| FSDT-test | *In vitro* (MOA) | Other *In vivo* | WoE | Next steps | Other |
|---|---|---|---|---|---|
| **+** | **0/Eq** | **-** | -effects-yes<br>-hormonal/advers? | Regulatory decision (?)<br>- Other MOA? | -test design for RA?<br>-FFLCT/MMLC |
| **+** | **0/Eq** | **0/Eq** | -effects-yes<br>-hormonal/advers? | Regulatory decision (?)<br>- Other MOA? | -test design for RA?<br>-FFLCT/MMLC<br>-In vitro- MOA |
| - | **+** | **+** | EDC but not in fish | Regulatory decision (?) | - Fish screens ? |
| - | **+** | **-** | -EDC (?)<br>-Degradation of ED | Regulatory decision (?) | - Fish screens ? |
| - | **+** | **0/Eq** | -EDC (?)<br>- nor covered by FSDT | Not an ED in fish(?) | - Fish screens ? |
| - | **?** | **?** | **?** | ? | ? |

# *GD on EDC assessment- Uterotrophic test OECD TG 440*

| TG 440 | In vitro (MOA) | Other In vivo | WoE | Next steps | Other |
|---|---|---|---|---|---|
| + | + | + | - strong evidence E/anti-E<br>- adverse effects? | Confirm with higher tier test (1-gen, 2-gen) | If positive data exists, confirm as EDC, consider exp/kinetics |
| + | + | - | potential E/anti-E activity via ER | Confirm with higher tier test (1-gen, 2-gen) | If negative data exists, confirm as no-EDC |
| + | - | + | - strong evidence E/anti-E<br>- metab.activation? | - In vitro with metabolic activation | - If positive data, evidence of concern and consider exp. |
| - | + | - | Weak evidence for E/anti-E activity. Acts via non-ER mechanism | In vitro with metabolic activation and possibly higher tier test | If negative data exists, absense of concern and consider exp. |
| + | 0/Eq | 0/Eq | E/anti-E activity of unknown potency metab.activation? | In vitro with metabolic activation and possibly higher tier test | Look for chemical analogs, find MoA |

# *Mahdolliset EDC kriteerit*

- Kriteerit kuten muillekin erityisen huolen aineille esim.:
  - Cat. 1: confirmed (in vivo)
  - Cat. 2:
    - B – suspected (in vivo)
    - B – potential (in vitro/in silico)

- EDC- määritelmän on oltava selkeä ja sovittu EU:ssa
  - Kriteerin tulee perustua aineen ominaisuuksiin ei potentiaaliseen riskiin.
  - EFSA ja PPP porukka eturivissä 2013?

# *EDC:n tunnistamisen hallinnolliset vaikutukset*

- REACH, jos aine tunnistetaan ”confirmed EDC”
  - *Art.57(f) kohdan mukaan SVHC kandidaatiksi*
  - *tarve uudelle liitteelle ? (PBT, CMR)*
- Kasvinsuojeludirektiivin mukaan ko. tehoainetta ei voi hyväksyä
  - ”*unless human exposure is negligible*”

## *International development and harmonization of methods*

# *Nordic co-operation*





## NORD –UTTE COORDINATION GROUP UNDER NKG FINANCED BY NMR

Nord-UTTE = *Nordic cooperation group for the development of test methods in toxicology and ecotoxicology*

NKG = Nordiska Kemikal Gruppen

NMR = Nordiska MinisterRådet

*International development and harmonization of methods*

# *Nordic co-operation – Nord UTTE*





CONSISTS OF NATIONAL COORDINATORS (TOX AND ECOTOX)

COORDINATION OF COMMENTS TOWARDS OECD AND EU

FINANCIAL SUPPORT AND STEERING OF EXPERIMENTAL WORK

BUDGET AROUND1,5 MILLION DANISH KR/YEAR

NORDIC NETWORK MEETINGS BETWEEN RESEARCHERS AND REGULATORS



WWW.NORD-UTTE.ORG