GERMAN FEDERAL INSTITUTE FOR RISK ASSESSMENT

BfR
Bundesinstitut für Risikobewertung

# Endocrine Disruptors – regulatory use of *in silico* as part of alternative methods

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# Endocrine disruptors and *in silico* methods

## Outline

- Regulatory Background Biocides and Plant Protection Products
- Guidance Documents on ED Assessment
- Experience in the EU
- Further Developments

# ED identification in plant protection and biocidal products

**“Cut-off criteria” for active substances in Plant Protection and Biocidal Products**

When an active substance in a **plant protection product** or a **biocidal product** is identified as an **ED** it leads to the **ban** of the substance from the market **unless**:

**Biocides**

- **negligible risk** is demonstrated
- essential substance to prevent/control serious danger
- dispoportional negative impact on society

**Plant protection products**

- **negligible exposure** under realistic conditions is demonstrated

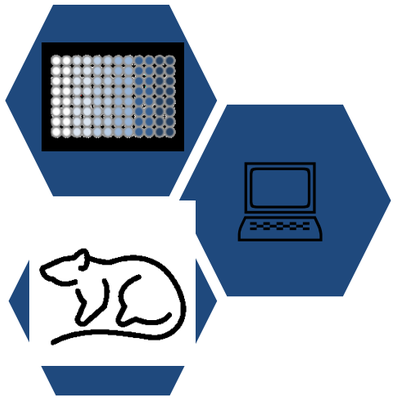
# ED identification in plant protection and biocidal products

**Biocidal products (Reg. (EU) 2017/2100 applicable from 7 June 2018)**
**Plant protection products (Reg. (EU) 2018/605 applicable from 10 November 2018)**

1. Adverse effect
2. Endocrine mode of action
3. The adverse effect is a consequence of the endocrine mode of action (plausible link between the two)

Identification is based on a **Weight of Evidence approach** considering following factors:

- both positive or negative results,
- relevance of study design,
- quality of data,
- toxicokinetics,
- limit dose,
- consistency/coherence,
- route of exposure,
- metabolism.



# ED identification in plant protection and biocidal products

**WHO/IPCS Definition of endocrine disruptors (2002)**:

“An exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations”

**WHO/IPCS Definition of Adversity (2004)**:

“A change in morphology, physiology, growth, development or lifespan of an organism which results in impairment of functional capacity or impairment of capacity to compensate for additional stress or increased susceptibility to the harmful effects of other environmental influences”

# EFSA/ECHA ED Guidance Document

**Developed by:**
- EFSA/ECHA Joined team
- Support from JRC

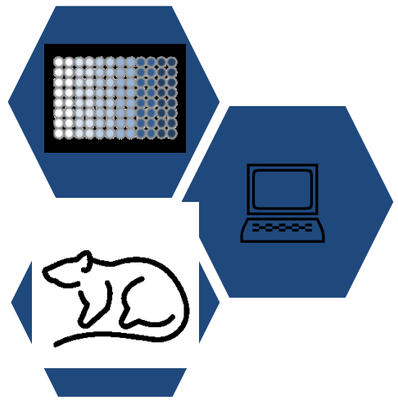
**Developed for:**
- Competent authorities
- Applicants

**In consultation with:**
- Public
- Stakeholders
- Member States experts
- Risk assessors
- Risk managers
- EFSA Scientific Committee
- EFSA Panel on PPP
- ECHA ED Expert Group

# EFSA/ECHA ED Guidance Document

➢ **The Assessment strategy is set up to use the available data efficiently!**

- **ED assessment starts with the data available**
- **Dataset must however be compliant with the information requirements of the Biocidal and Plant Protection Products Regulation and is therefore mainly focused on *in vivo* and *in vitro* tests**
- **Generation of further data *(in particular of animal studies)* only when necessary**
- **Available information on mammals/humans and on non-target organisms is used holistically in the assessment**



# EFSA/ECHA ED Guidance Document

**Focused on:**

- Estrogen, androgen, thyroid, steroidogenic modalities (EATS)
- vertebrates (human health & environment)

**Assessment strategy based on:**

- ED Scientific Criteria
- OECD conceptual framework
- OECD GD 150

**Applicable under:**

- EU PPP Regulation
- EU BP Regulation

# EFSA/ECHA ED Guidance Document

- **ED criteria** for Biocides and Plant Protection Products
- **OECD conceptual framework** for testing and assessment of endocrine disruptors
  - Lists the OECD test guidelines and other standardised test methods which can be used to evaluate substances for endocrine disruption
  - Provides guidance on the use of the test methods, but is not a testing strategy
- **OECD guidance document 150** on standardised test guidelines for evaluation of endocrine disruption
  - Helps the interpretation of results for the **parameters (~ effects)** investigated in the assays available for ED testing

# EFSA/ECHA ED Guidance Document: EATS modalities

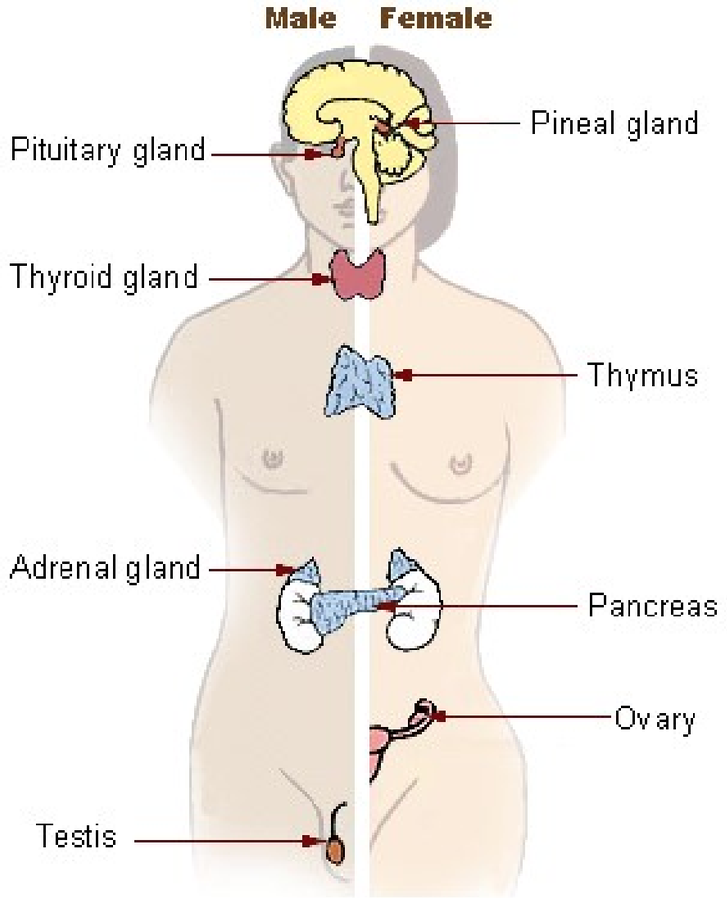
**Hypothalamus/Pituitary**
- Oxytocin
- Vasopressin (ADH)
- Somatotropin (GH)
- Prolactin (PRL)
- Follicle-stimulating hormone (FSH)
- Luteinizing hormone (LH)
- **Thyrotropin (TSH)**
- Adrenocorticotropin (ACTH)

**Thyroid gland**
- **Triiodothyronine (T3)**
- **Thyroxin (T4)**

**Parathyroid**
- Calcitonin
- Parathyrin (PTH)

**Adrenal gland**
- Adrenalin
- Noradrenalin
- **Glucocorticoids (e.g. Cortisol)**
- **Mineralocorticoids (e.g. Aldosterone)**

**Testes (Males)**
- **Androgens (e.g. Testosterone)**




https://en.wikipedia.org/wiki/Endocrine_system

**Dermis**
- Cholecalciferol (Vitamin D3)

**Thymus**
- Thymosin

**Adipose tissue**
- Leptin, Estrogens

**Stomach**
- Gastrin, Ghrelin, Secretin, Somatostatin, Histamine, Endothelin

**Kidney**
- Renin, Erythropoietin (EPO), Calcitriol, Thrombopoietin

**Pancreas**
- **Insulin**
- **Glucagon**

**Ovaries (Females)**
- **Estrogens (e.g. Estradiol)**
- **Gestagens (e.g. Progesterone)**

# EFSA/ECHA ED Guidance Document: OECD Conceptual Framework

| | **Mammalian toxicology** |
|---|---|
| **Level 1**<br>**Existing Data and**<br>**Non-Test Information** | • Phys-chem. properties, e.g. MW, reactivity, volatility<br>• All available toxicological data from standardised and non-standardised tests<br>• **Read-across, chemical categories, QSARs, other *in silico* prediction, ADME modelling** |
| **Level 2**<br>***In vitro* assays (selected endocrine mechanism(s)/pathway(s))** | • Oestrogen or androgen receptor binding affinity<br>• Oestrogen receptor transactivation (OECD TG 455, 457)<br>• Androgen or thyroid transactivation<br>• Steroidogenesis in vitro (OECD TG 456)<br>• Other assays as appropriate |
| **Level 3**<br>***In vivo* assays (selected endocrine mechanism(s)/pathway(s))** | • Uterotrophic assay (OECD TG 440<br>• Hershberger assay (OECD TG 441)) |
| **Level 4**<br>***In vivo* assays (adverse effects on endocrine relevant endpoints)** | • Repeated dose 28-day study (OECD TG 407)<br>• Repeated dose 90-day study (OECD TG 408<br>• 1-gen reproductive toxicity study (OECD TG 415)<br>• Prenatal dev toxicity study (OECD TG 414)<br>• Chronic toxicity and carcinogenicity studies (OECD TG 451-453)<br>• Developmental neurotoxicity (OECD TG 426) |
| **Level 5**<br>***In vivo* assays (comprehensive data on adverse effects on endocrine relevant endpoints over more extensive parts of the life cycle)** | • Extended one-gen. reproductive toxicity study (OECD TG 443)<br>• 2-gen. reproduction toxicity study (OECD TG 416, most recent update) |

# EFSA/ECHA ED Guidance Document: *in silico*

„Whenever *in silico* methods are used, the general provisions outlined in ECHA Guidance R6 should be followed (ECHA, 2008).“

The different types of *in silico* methods:

1. Molecular modelling of receptor interactions:
   - Prerequisites:
     - precise knowledge about receptor structures,
     - presteps of selection of „active“ conformers, or
     - supercomputers
   - less likely to be used routinely in regulatory processes
   - useful for supporting the identification of MoA

2. (Q)SAR modelling of receptor-based activity:
   - mathematical relations between structural and/or phys-chem properties of chemicals and receptor-related effects or downstream effects:
     - Agonist/antagonist modelling
     - Qualitative and quantitative binding information
   - easy to use, esp. when implemented in software

3. Profilers based on structural alerts and decision trees:
   - support of grouping and read-across
   - based on existing SARs or chemotypes
   - easy to use, typically implemented in software

# EFSA/ECHA ED Guidance Document: *in silico*

- (Q)SAR
- Molecular docking

**Table 11:** Software tools for predicting endocrine activity

| Software tool | Effect addressed | | | |
|---|---|---|---|---|
| | E | A | T | S |
| EDKB | X | X | | |
| ADMET Predictor | X | | | |
| ACD/Labs Percepta – Toxicity Module | X | | | |
| Derek | X | | | |
| MolCode Toolbox | X | | | |
| CASE Ultra | X | X | | |
| TIMES | X | X | | |
| VirtualToxLab | X | X | X | X(b) |
| OECD (Q)SAR Toolbox | X | | | |
| Endocrine Disruptome | X | X | X | X(d) |
| COSMOS KNIME workflow | X | X | X | X(d) |
| Danish (Q)SAR DB | X | X | X | |
| (Q)SAR Data Bank | X | | | |
| VEGA platform | X | | | |
| + CERAPP (based on ToxCast | X | | | |

**E:** estrogen receptor α/β

**A:** androgen receptor

**T:** thyroid receptor α/β

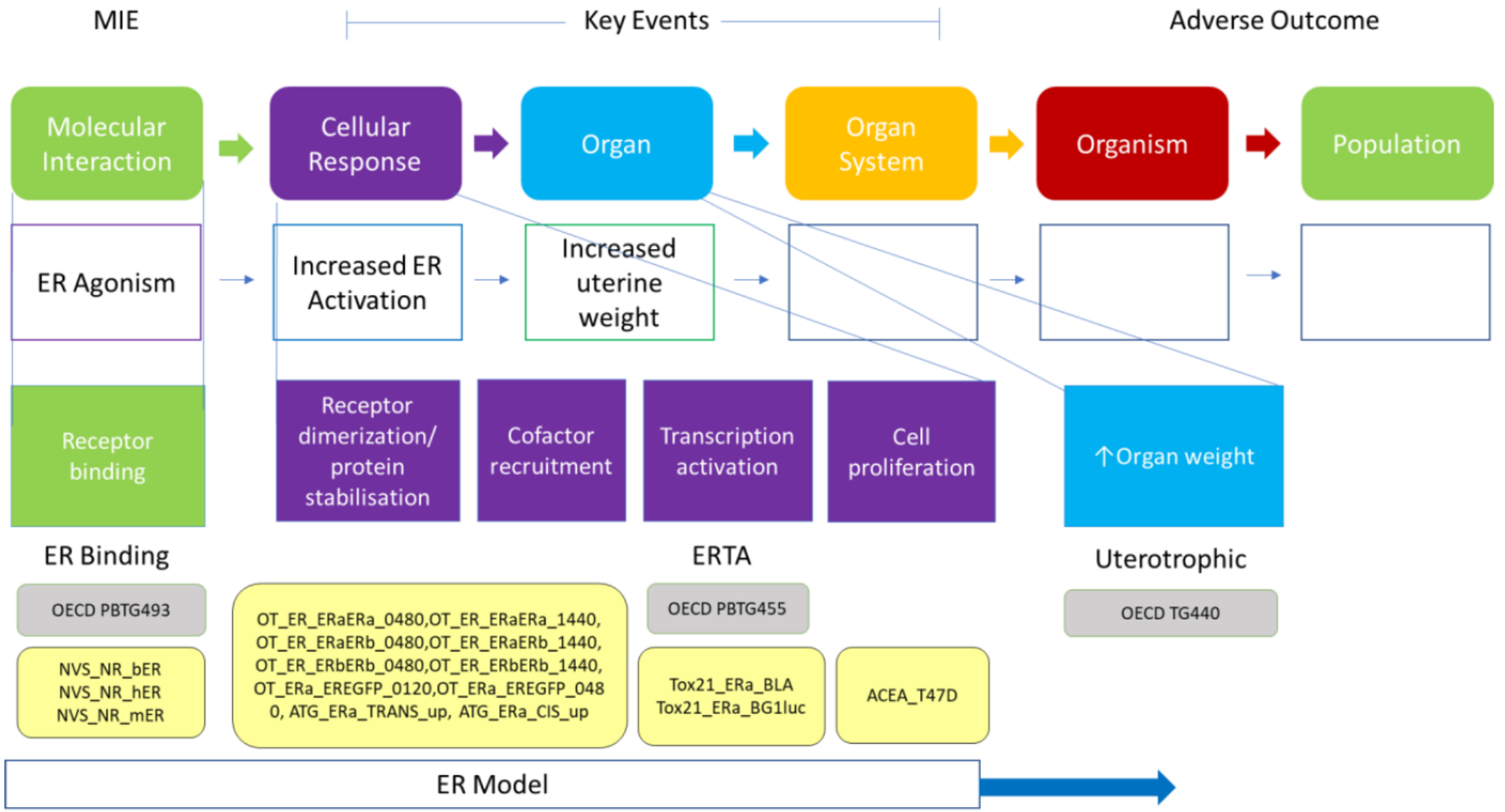
**S:** (b): glucocorticoid receptor, mineralocorticoid receptor
(d): glucocorticoid receptor

# EFSA/ECHA ED Guidance Document: *in silico*

## Content – Appendices:

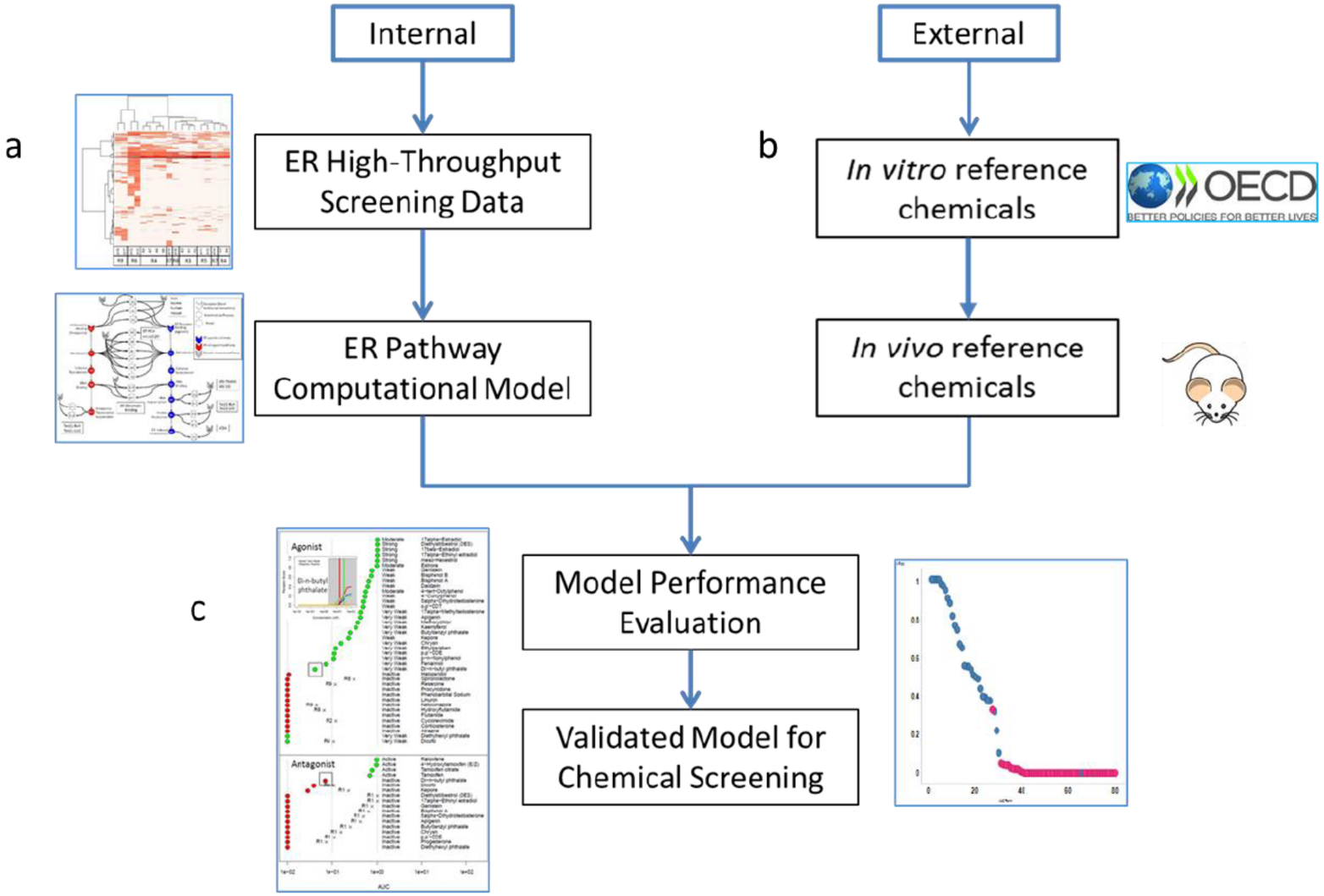
| | |
|---|---|
| APPENDIX A | **Additional considerations on how to asses the potential for thyroid disruption in human health** |
| **APPENDIX B** | **Recommendations for design, conduction and technical evaluation of hormonal studies** |
| APPENDIX C | **Information requirements for active substances under the Biocidal Products and Plant Protection Products Regulations, which could potentially provide information on endocrine disrupting properties** |
| APPENDIX D | **Databases, software tools and literature-derived (Q)SARS** |
| APPENDIX E | **Excel template for reporting the available information relevant for ED assessment** |
| APPENDIX F | **Example of how to develop the search strategy protocol** |
| APPENDIX G | **Example of Mode of Action (MoA) for non-target organisms (Fish)** |

**Figure 1. Representation of the ER pathway and computational model in the context of the molecular initiating event (MIE) and associated key events (adapted from Browne et al. 2017).**




CASE STUDY ON THE USE OF AN INTEGRATED APPROACH TO TESTING AND ASSESSMENT FOR ESTROGEN RECEPTOR ACTIVE CHEMICALS
Series on Testing and Assessment No. 309, OECD, 2019

**Figure 2. Conceptual diagram of the overall process for development, validation, and application of the IATA.**

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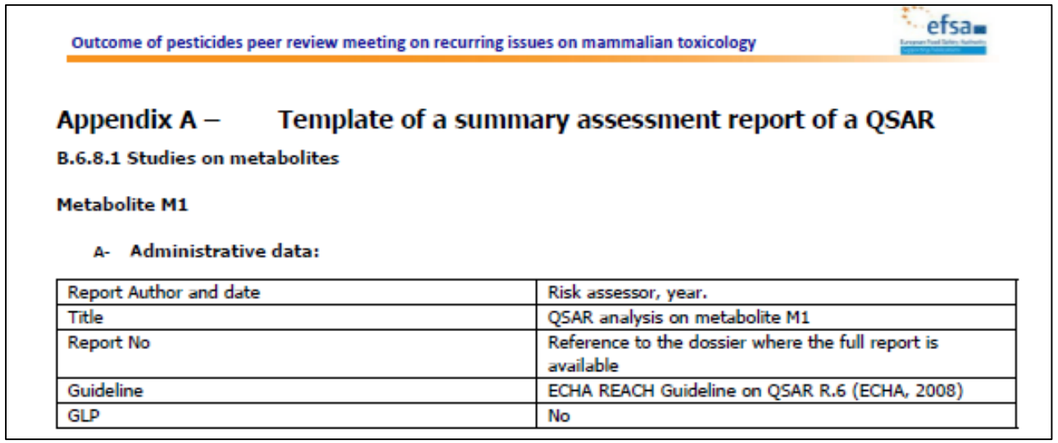
# EFSA/ECHA ED Guidance Document: *in silico*

## Experiences so far:

- consideration of applicability domain
- only receptor binding and profilers/read-across at the moment
- useful for decisions on mechanistic level 2/level 3 studies
- harmonised reporting format required
  (adaptation from QSAR reporting format for
  metabolite genotoxicity in PPP)



Outcome of pesticides peer review meeting on recurring issues on mammalian toxicology

efsa

**Appendix A – Template of a summary assessment report of a QSAR**

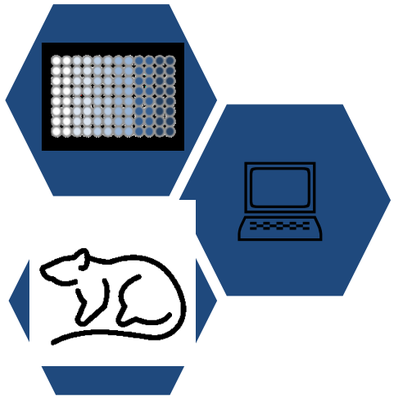
**B.6.8.1 Studies on metabolites**

**Metabolite M1**

**A- Administrative data:**

| | |
|---|---|
| Report Author and date | Risk assessor, year. |
| Title | QSAR analysis on metabolite M1 |
| Report No | Reference to the dossier where the full report is available |
| Guideline | ECHA REACH Guideline on QSAR R.6 (ECHA, 2008) |
| GLP | No |

EFSA Supporting Publication 2020-EN-1837



# Further developments

E, A, S:

Steroid hormone synthesis

Aromatase inhibition

T

Thyroid hormone synthesis

TPO inhibition

NIS inhibition

Thyroid hormone transfer

Inhibition of

OATP1C1

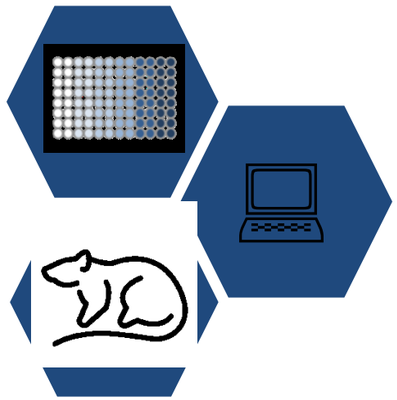
MCT8

DIO1, DIO2, DIO3

# Further Developments

EURION: 8 Horizon2020 projects to improve the identification of endocrine disruptors

- ATHENA: focus on thyroid, including QSAR development
- EDCMET: focus on metabolic disorders, including *in silico*
- ENDpoiNTs: focus on developmental neurotoxicity, testing methods
- ERGO: use of non-mammalian vertebrate research for human health
- FREIA: AOPs for female reproductive toxicology, including use of *in silico*
- GOLIATH: focus on metabolic disorders, including *in silico*
- OBERON: focus on metabolic disorders, including *in silico*
- SCREENED: focus on thyroid, based on *in vitro* testing



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# Thank you for your attention

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