Health and Safety
Executive

HSE

Assessment and regulation of endocrine disrupters under European chemical legislations

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EDs and the current EU legislative framework

- **PPP Regulation (1107/2009):**

  ED substance cannot be approved unless exposure negligible

- **Draft BP Regulation (COM(2009)267):**

  ED substance cannot be approved unless exposure negligible

- **REACH (1907/2006):**

  ED substance may be included in the list of substances subject to authorisation

Implications of these legislative stipulations

- Serious regulatory consequences of this hazard-based property being assigned to a substance;
- Important to ensure that the “ED” identifier is applied in a justified and discriminatory manner

EDs: available definitions

- Kavlock, 1996
- NRDC, 1998
- Weybridge, 1996

- **WHO/IPCS, 2002:**

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

UK proposal

- **WHO/IPCS definition: starting point**

  Still very broad description not able to discriminate between EDs of regulatory concern and EDs of low priority

- **Further elaboration for regulatory use and application**

  Add criteria of regulatory importance

WHO/IPCS (2002) ED definition – 2 key elements

- **Adverse effects via endocrine perturbation**

  Endocrine perturbation resulting in pathology or functional impairment

- **Adverse effects observed in an intact organism**

  Whole animal observations

Additional criteria of regulatory importance

(1) Adverse effects raising a concern for ED to have been seen in one or more toxicity studies of acceptable quality:

- Relevant routes of exposures;
- Follow general scientific principles in design, conduct and reporting;
- Appropriate statistics

Additional criteria of regulatory importance

(2) Adverse effects raising a concern for ED to be the lead toxic effect in the dataset or occurring at a dose level close to that at which the lead toxic effect was first seen:

- Most sensitive effect; or
- Occurring in the same dose range (up to 3-fold the dose of the most sensitive effect) producing other significant/major effects

Additional criteria of regulatory importance

(3) Potency considerations – serious adverse effects raising a concern for ED to have been produced at relevant dose levels:

Serious adverse effects ≤ STOT-RE Category 2 guidance values of the CLP Regulation

	Sub-acute	Subchronic	Chronic
Oral	300 mg/kg bw/day	100 mg/kg bw/day	50 mg/kg bw/day
Dermal	600 mg/kg bw/day	200 mg/kg bw/day	100 mg/kg bw/day
Inhalation (vapour	3 mg/l/6h/day	1 mg/l/6h/day	0.5 mg/l/6h/day
Inhalation (dust/mist/fume)	0.6 mg/l/6h/day	0.2 mg/l/6h/day	0.1 mg/l/6h/day

Additional criteria of regulatory importance

(4) A mode-of action link between the adverse effects and endocrine disruption to have been established:

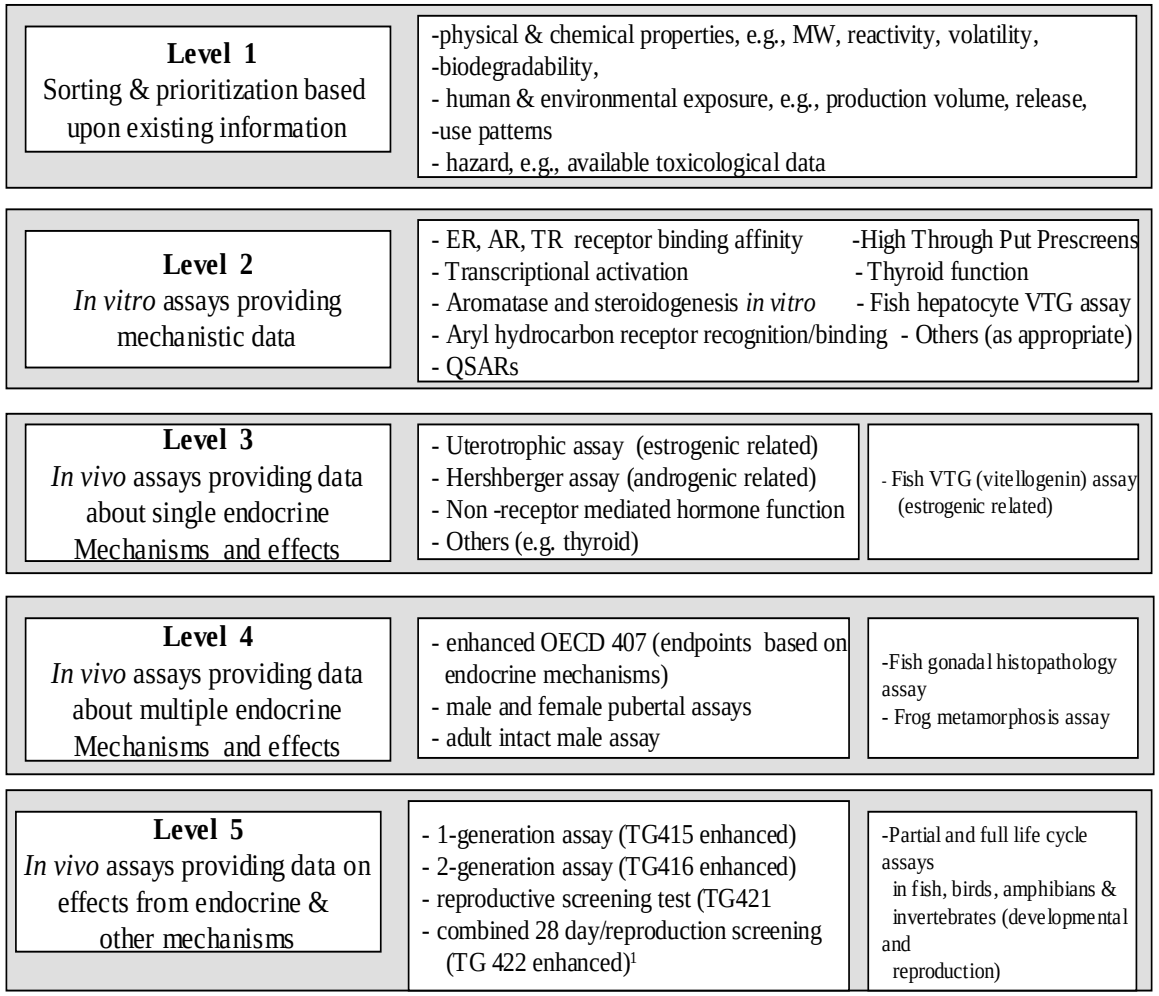
- In vitro and in vivo screening assays (level 2 to 4) of the OECD conceptual framework for testing and assessment of EDs;
- Other more ad-hoc mechanistic studies;
- If such studies not available, should be requested

Additional criteria of regulatory importance

(5) The effects seen in experimental animals to be of potential relevance to human health:

- Use IPCS human relevance framework for robust and transparent conclusion;
- If no information, assume relevance

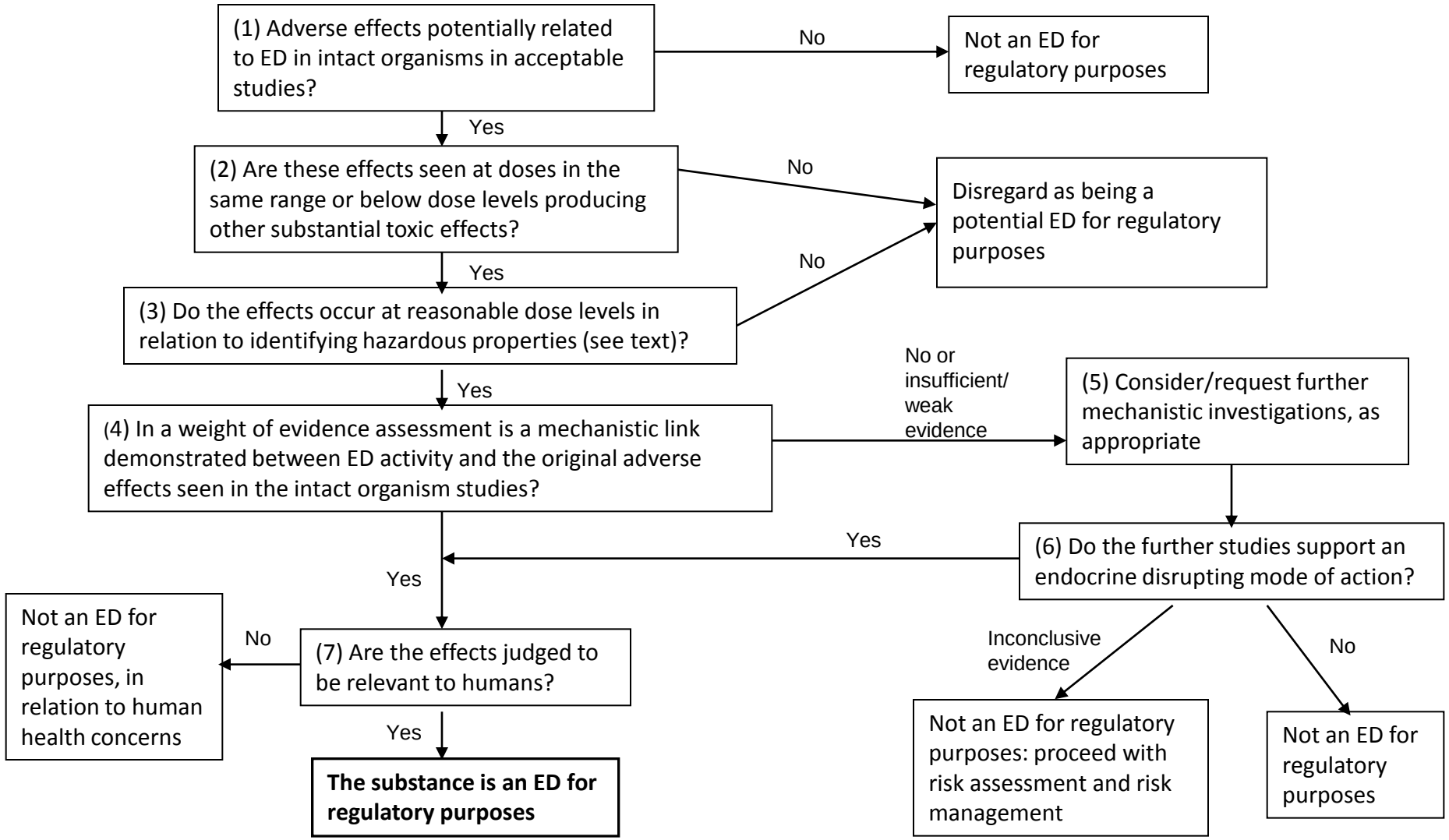
OECD conceptual framework for the testing and assessment of EDs



OECD ED Level 2 - 4 assays

	Assay	Information provided
Level 2 In vitro assays providing mechanistic data	-ER, AR, TR receptor binding affinity - Transcriptional activation - Aromatase and steroidogenesis in vitro -AhR receptor binding	-Binding to oestrogen, androgen and thyroid receptor - Alterations in the synthesis of sex steroid hormones - Inhibition of aromatase
Level 3 In vivo assays providing data about single endocrine mechanisms and effects	-Uterotrophic assay - Hershberger assay - Others (thyroid)	- Oestrogenic, androgenic, anti-androgenic and anti-thyroid activity
Level 4 In vivo assays providing data about multiple endocrine mechanisms and effects	-Enhanced OECD 407 - Male and female pubertal assays - Adult intact male assay	- Alterations in pubertal development via changes in gonadotrophins, prolactin or hypothalamic function

Decision tree to establish whether substance is an ED for regulatory purposes in relation to human health



Case study: vinclozolin
Application of the decision tree (1)

(1) Adverse effects potentially related to ED in intact organisms?

Yes, V causes Leydig cell tumours, atrophy of accessory sex glands, malformations in the male urogenital tract, feminisation, uterine and ovary tumours;

(2) Most sensitive/lead effect?

Yes;

(3) Relevant dose levels?

Yes, 100% hypospadias and 100% nipple retention at 100 mg/kg bw/day in dev tox studies < STOT-RE 2 of 300 mg/kg bw/day;

(4) Endocrine mechanistic link?

Yes, V binds to the androgen receptor and causes inhibition of transcription of androgen-dependent genes. Positive results in AR binding affinity and transcriptional activation tests in vitro and in the Hershberger assay and male pubertal assay in vivo

Case study: vinclozolin
Application of the decision tree (2)

(5,6) Consider further mechanistic investigations, as appropriate

No further testing is necessary – there is sufficient evidence for a robust conclusion;

(7) Are the effects relevant to humans?

Yes – the toxicokinetic and toxicodynamics of V are expected to be similar in rats and humans

<u>Vinclozolin is an ED for regulatory purposes, in relation to human health</u>

Case study: 1,3-DNB
Application of the decision tree (1)

(1) Adverse effects potentially related to ED in intact organisms?

Yes, 1,3-DNB causes testicular toxicity in the adult rat;

(2) Most sensitive/lead effect?

Yes, testicular toxicity occurs in the same dose range as other toxic effects (haematological effects and decreased body weights);

(3) Relevant dose levels?

Yes, testes atrophy from 6.6 mg/kg bw/day in 8-wk study < STOT-RE 2 of 100 mg/kg bw/day;

(4) Endocrine mechanistic link?

No, 1,3-DNB is negative in the steroidogenesis assay, has no androgen or oestrogen binding activity (QSAR); also no changes in serum LH, FSH and prolactin in vivo

Case study: 1,3-DNB
Application of the decision tree (2)

(5,6) Consider further mechanistic investigations, as appropriate

No further testing is necessary – there is sufficient evidence for a robust conclusion;

<u>1,3-DNB is not an ED for regulatory purposes, in relation to human health</u>

What is now happening with the UK proposal?

- Been endorsed by COT
- Have compared it to those of others, e.g. BfR, ECETOC;
- Have sent out on limited targetted consultation;
- Triazoles exercise in relation to PPPs;
- Continue to debate and reflect on during Autumn-Winter 2010