ASSESSING COSMETIC INGREDIENTS FOR ENDOCRINE DISRUPTING PROPERTIES

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THINKING AHEAD IN TOXICOLOGY

Introduction

In the EU Cosmetics Regulation, there are no specific provisions addressing endocrine disruptors (ED) but a fitness check conducted by the European Commission (COM(2018)739) concluded that the tools foreseen in the Regulation are suitable to deal with potential health hazards connected with ED. In 2014, the EU Scientific Committee on Consumer Safety (SCCS) adopted a memorandum explaining the assessment process for potential ED. It also specified that the substances should be treated like other substances of concern for human health, therefore, be subject to risk assessment.

The objective of the poster is to present a stepwise ED assessment approach which considers the information derived from New Approach Methodologies (e.g., (Q)SAR, read across and in vitro assays). This approach is in line with the principles laid down in the OECD Conceptual Framework for the testing and assessment of endocrine disruptors and in the ECHA/EFSA 2018 guidance for the identification of EDs under the EU Biocides and Pesticides Regulation.

ED assessment strategy for cosmetic ingredients

Compilation and analysis of available information

Level 1

- Literature search to identify existing data
  - *In silico* predictions for binding to receptors, hormones or enzymes
- ED-specific databases (e.g., EASIS, TEDX list, NUSRA, EDKB and EADB of FDA)
  - OECD Toolbox, Derek® Nexus, Endocrine disruptome, ToxCast models, Danish QSAR database (DB) and VEGA

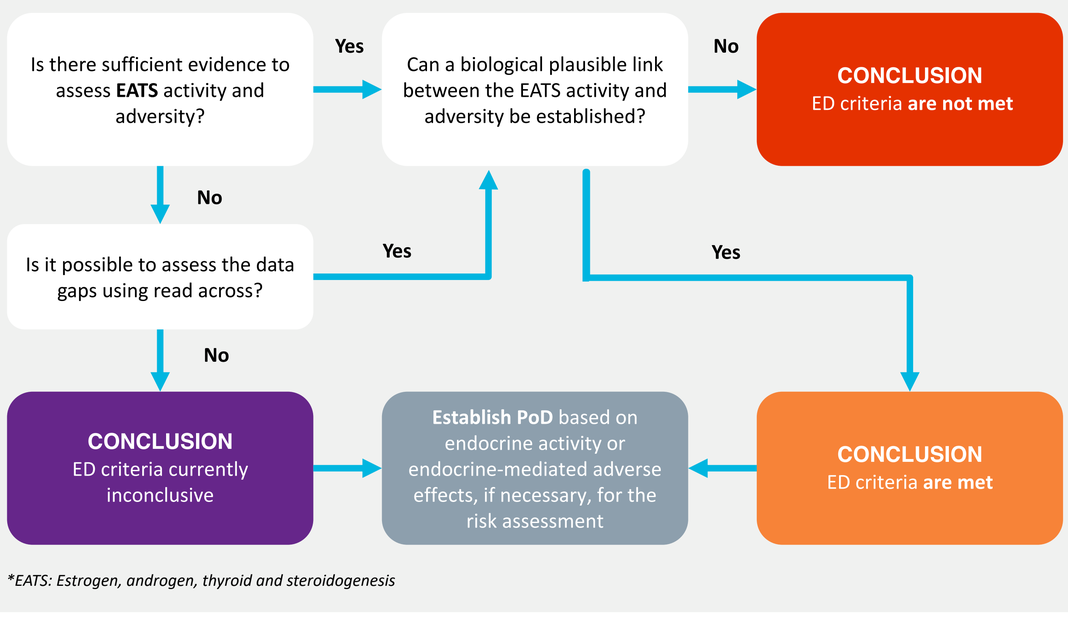
Level 2 & 3

- Existing *in vitro* mechanistic assays
  - Existing *in vivo* mechanistic assays
- *In vitro* high throughput screening (HTS) assays from ToxCast/PubChem and literature
  - Uterotrophic assay (OECD 440), Hershberger assay (OECD 441)

Level 4 & 5

- Existing *in vivo* repeated dose toxicity studies
  - Existing *in vivo* reproductive toxicity studies
  - Existing *in vivo* development toxicity studies
- Subacute (e.g., OECD 407), subchronic (e.g., OECD 408) or chronic toxicity studies (e.g., OECD 451-453)
  - Screening reproductive and development toxicity (e.g., OECD 421/422), 2-generation reproductive toxicity (e.g., OECD 416) studies
  - Pre-natal development toxicity OECD 414), Developmental neurotoxicity (OECD 426) studies

Assessment of EATS* modalities and Point of Departure selection



**EATS: Estrogen, androgen, thyroid and steroidogenesis*

Figure 1: ED assessment framework for cosmetic ingredients

Case Study I: Data rich substance

OBJECTIVE

SCCS performed an assessment for octocrylene (CAS No. 6197-30-4) due to a suspected ED concern for its use in cosmetic formulations.

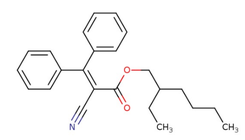
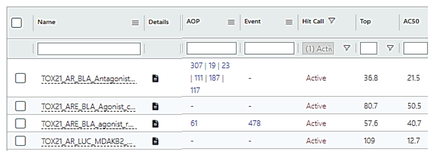


Figure 2: *Octocrylene (CAS No. 6197-30-4)*



Name	Details	AOP	Event	Hit Call	Top	AC50
TOX21_AR_BLA_Antagonist...		307 | 19 | 23 | 111 | 187 | 117	-	Active	36.8	21.5
TOX21_ARE_BLA_Agonist_c...		-	-	Active	80.7	50.5
TOX21_ARE_BLA_agonist_r...		61	478	Active	57.6	40.7
TOX21_AR_LUC_MDAKB2_...		-	-	Active	109	12.7

Figure 3: *ToxCast results*

COMPILATION OF INFORMATION AND ASSESSMENT OF EATS MODALITIES

OECD Level	Available information/studies
Level 1 (*non-test information*)	• *In silico* predictions: DEREK (no alerts); Danish EPA QSAR DB (no binding to ER, AR, TPO); VEGA (no EATS activity); Endocrine disruptome (AR_{an} and TR binding potential); ToxCast models (very weak to weak E binding and activity) → Some EAT activity
Level 2 (*in vitro* ED activity)	• ToxCast assays: Active in 7 (E=3; A=2; T=1; S=1) out of 19 ED-relevant assays. AC50 of all 7 assays exceed the cytotoxic concentration → non-specific EATS activity • *In vitro* yeast human ER and AR transactivation assays: anti-androgenic/androgenic activity – effects not considered relevant for *in vivo* situation → A activity inconclusive • *In vitro* assay in human sperm cells: no impact on sperm functions → no A activity
Level 3 (*in vivo* ED activity)	• *In vivo* uterotrophic assay: No effects → no E activity • *In vivo* Hershberger assay: No effects → no A activity
Levels 4 & 5 (*in vivo* assays on ED adversity)	• **RDT studies:** No effects on EATS-mediated (e.g., gonads weight (wt) and histopathology including thyroid) or EATS 'sensitive but not diagnostic' parameters (e.g., adrenal wt and histopathology; NOAEL: 175 mg/kg bw/day in rats (oral); 534 mg/kg bw/day in rabbits (dermal) → no EATS adversity • **EOGRT study:** ↑ thyroid wt. and histopathological changes (due to ↑ GGT *adaptive change*); slight ↑ in preputial separation, vaginal opening or 1st estrous stage in cohort 1A offspring (due to ↓ pup wt)→ No effects on the majority of EATS-mediated parameters (e.g., gonads, TSH and T4), except for effect on sexual development → **NOAEL = 153/163 mg/kg bw/day** in rats (oral) • **PND studies:** No effects on EATS-mediated (uterus and thyroid wt and histopathology) or EATS 'sensitive but not diagnostic' parameters (e.g., gestation length, litter size/wt/viability, implantation loss, sex ratio and resorption) → NOAEL = 1000 mg/kg bw/day in rats (oral); 267 mg/kg bw/day (HD) in rabbits (dermal); 1000 mg/kg bw/day in mice (oral) → no EATS adversity

AC50: concentration showing 50% activity; AR: androgen receptor; EOGRT: Extended one generation reproductive toxicity; ER: estrogen receptor; GGT: Gamma glutamyl transferase; RDT: Repeated dose toxicity; PND: Pre-natal development toxicity; TPO: Thyroid peroxidase; TR: Thyroid receptor

ED ASSESSMENT AND SELECTION OF POD FOR RISK ASSESSMENT

No sufficient evidence to establish a biologically plausible link between ED activity and ED adversity.

NOAEL = 153 mg/kg bw/day based on the age of sexual development in the key EOGRT study considered as PoD.

CONCLUSION

Although no firm conclusions on ED could be made, the absence of a significant risk was concluded at the assessed concentration in the cosmetic formulation.

Case study II: Data poor substance

OBJECTIVE

A novel cosmetic ingredient was assessed for its toxicological properties, including ED and a risk assessment for use in a cosmetic formulation.

COMPILATION OF INFORMATION AND ASSESSMENT OF EATS MODALITIES

OECD Level	Available information/studies
Level 1 (*non-test information*)	• *In silico* predictions: DEREK (no alerts); Danish EPA QSAR DB (no binding to ER, AR and TPO); VEGA (no EATS activity); Endocrine disruptome (AR_{an} binding potential (low-medium probability); ToxCast models (inactive E and A binding and activity) → No significant EATS activity
Level 2 (*in vitro* ED activity)	• ToxCast assays: Active in 7 (E=3; A=2; T=1; S=1) out of 19 ED-relevant assays. Some ER and AR agonistic activity at concentrations ≥20 µM (equivalent to 5-7 mg/kg bw/day using NTP IVIVE tool with default settings)~~;~~ → some E and A activity
Level 3 (*in vivo* ED activity)	• No data
Levels 4 & 5 (*in vivo* assays on ED adversity)	• **RDT study:** No effects on EATS-mediated parameters (e.g., gonads and thyroid wt. and histopathology) or endocrine sensitive but not diagnostic parameters (e.g., adrenal wt.) parameters in a 90-day study in rats (oral); NOAEL: 1000 mg/kg bw/day → no EATS adversity • **PND study:** No effects on EATS-M (e.g., uterus wt) or ESnD (e.g., fetal development, pup wt., implantations loss) in rabbits; NOAEL = 288 mg/kg bw/day in rabbits→ no EATS adversity

ED ASSESSMENT AND SELECTION OF POD FOR RISK ASSESSMENT:

- No sufficient evidence to establish a biologically plausible link between ED activity and ED adversity.
- The 'equivalent administered doses' (EAD) in mg/kg bw/day derived from the in vitro ED assay using *in vitro* to *in vivo* extrapolation (IVIVE) interface of the NTP ICE tool, which was lower than the in vivo dose of 288 mg/kg bw/day from the PND study was considered as PoD for a preliminary risk assessment.
- For a more refined risk assessment, Physiological based pharmacokinetic (PBPK) modelling could be used to calculate the internal concentrations and refine the risk assessment.

CONCLUSION

Although no firm conclusions on ED could be made, the absence of a significant risk due to ED or any other hazard effects was concluded at the assessed concentration in the cosmetic formulation.

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