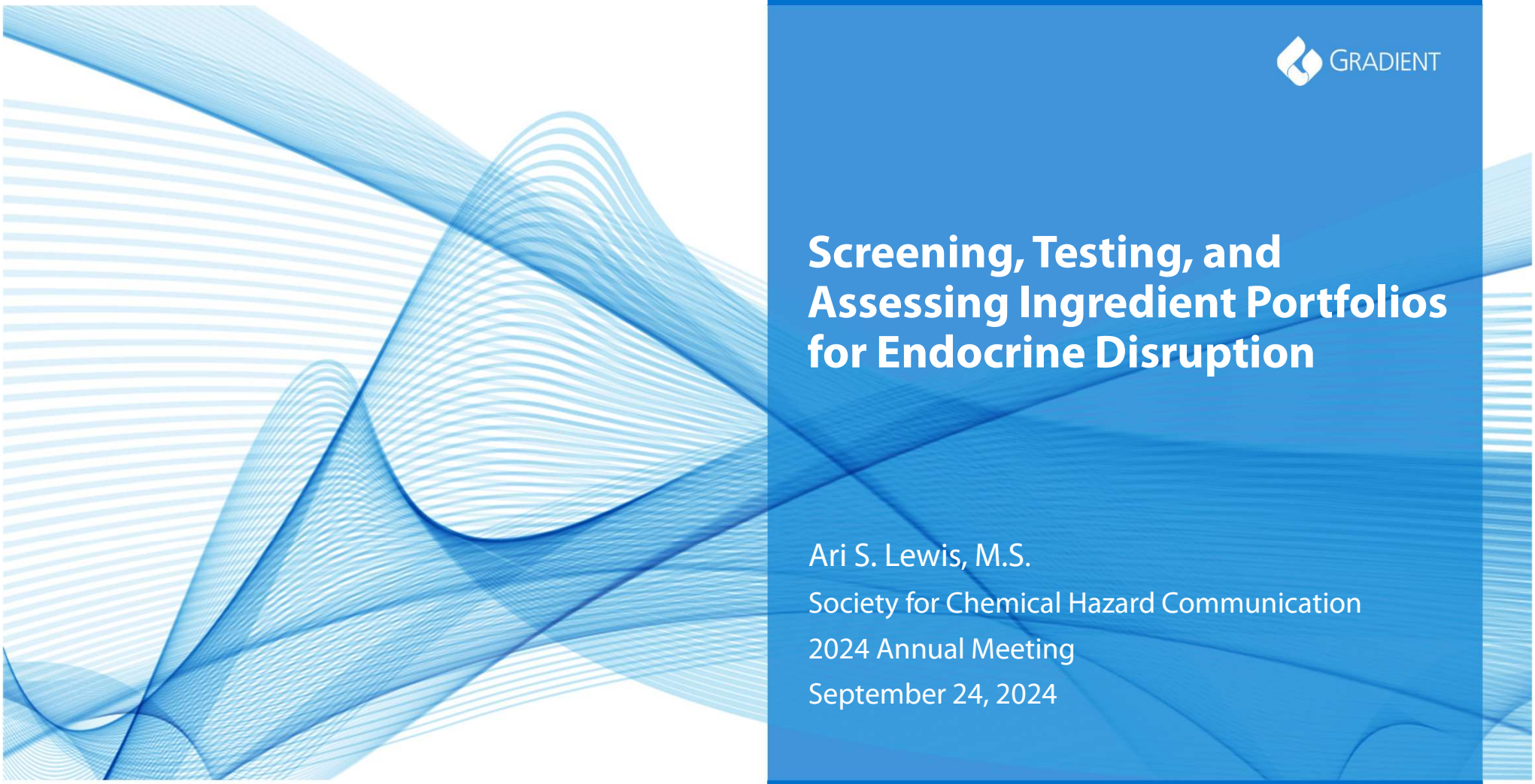

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# Screening, Testing, and Assessing Ingredient Portfolios for Endocrine Disruption

Ari S. Lewis, M.S.

Society for Chemical Hazard Communication
2024 Annual Meeting
September 24, 2024

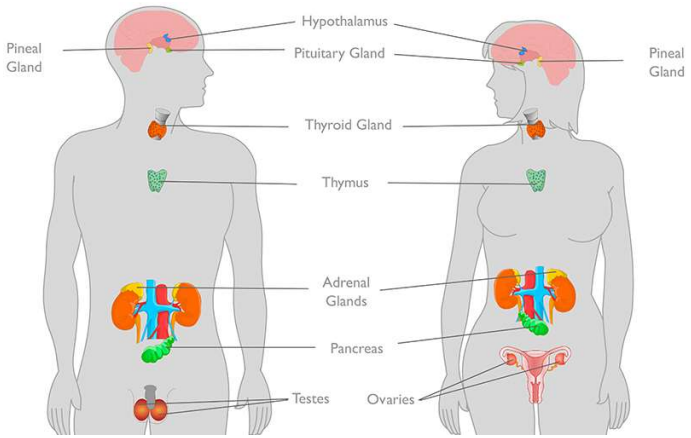
# Outline

- Endocrine disruption (ED) primer
- Regulatory background – European Union (EU)
- Recent EU regulatory developments:  new Classification, Labelling and Packaging (CLP) hazard classes
- Evaluating your ingredient portfolio in light of these developments

# What Is Endocrine Disruption?

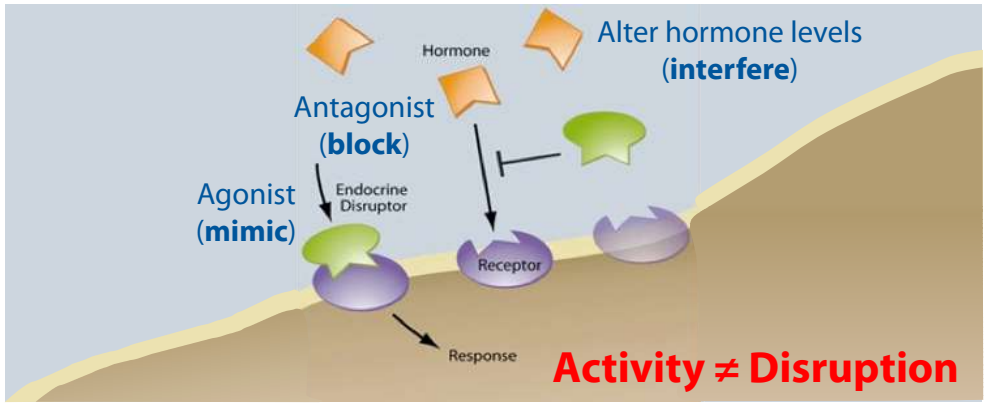
## Endocrine System

- A collection of glands that secrete chemical messengers and hormones that coordinate many functions (including reproduction) in target tissues and cells that contain hormone receptors




## Endocrine Disruption

- An endocrine disruptor is an exogenous substance or mixture that alters function(s) of the endocrine system and, consequently, causes adverse health effects in an intact organism, its progeny, or (sub)populations (WHO/IPCS, 2002; EC, 2022)



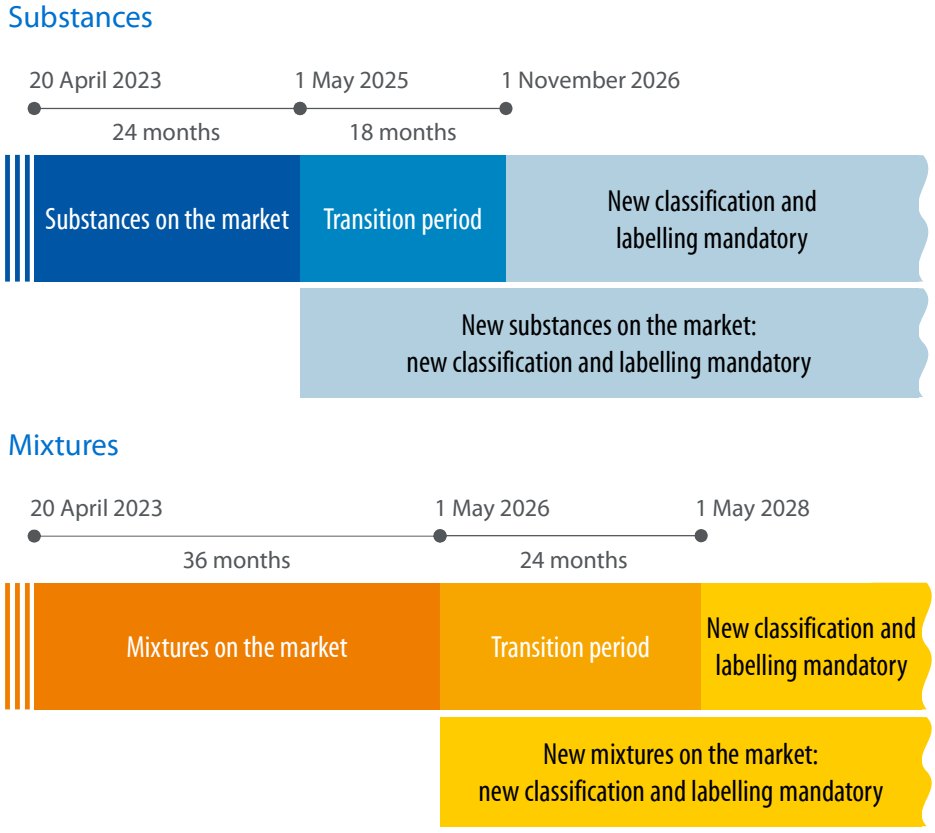

Focus on estrogen, androgen, thyroid, and steroidogenesis (EATS) modalities, but growing interest in non-EATS modalities (*e.g.*, retinoic acid, aryl hydrocarbon receptor, glucocorticoids)

# Regulatory Background: EU

- **1999:** European Commission (EC) adopted Community Strategy for EDs
  - Short-, medium-, and long-term actions
  - No mandatory testing program
- **Last Decade: Several Legislative Actions**
  - Biocidal Products (BP) Regulation and Plant Protection Products (PPP) Regulation
    - 2018 EFSA/ECHA guidance for the identification of EDs under BP and PPP Regulations
  - REACH
    - Substances of very high concern (SVHC); Annex XIV Authorization List
  - Water
    - Priority substances, environmental quality standard (EQS) development and monitoring
  - Cosmetics
    - Restrictions/bans on numerous ED preservatives
- **Currently:** Developing horizontal approach for identification and regulation of EDs ("one substance, one assessment" under the EU Green Deal and Chemicals Strategy for Sustainability)

# Recent Regulatory Developments in the EU: New CLP Hazard Classes

- Regulation (EC) No 1272/2008 on CLP of substances and mixtures
- New hazard classes proposed in 2022
  - Endocrine-Human Health & Environment
  - PBT/vPvB and PMT/vPvM
- Final guidance expected Fall 2024 (Draft version of guidance available July 2024)
- Transition period for reclassification and labelling of substances and mixtures
- **CLP Anticipated to Become a Key Regulatory Driver for ED in the Coming Years**




Source: ECHA (2023). https://echa.europa.eu/new-hazard-classes-2023.

# New Hazard Classes

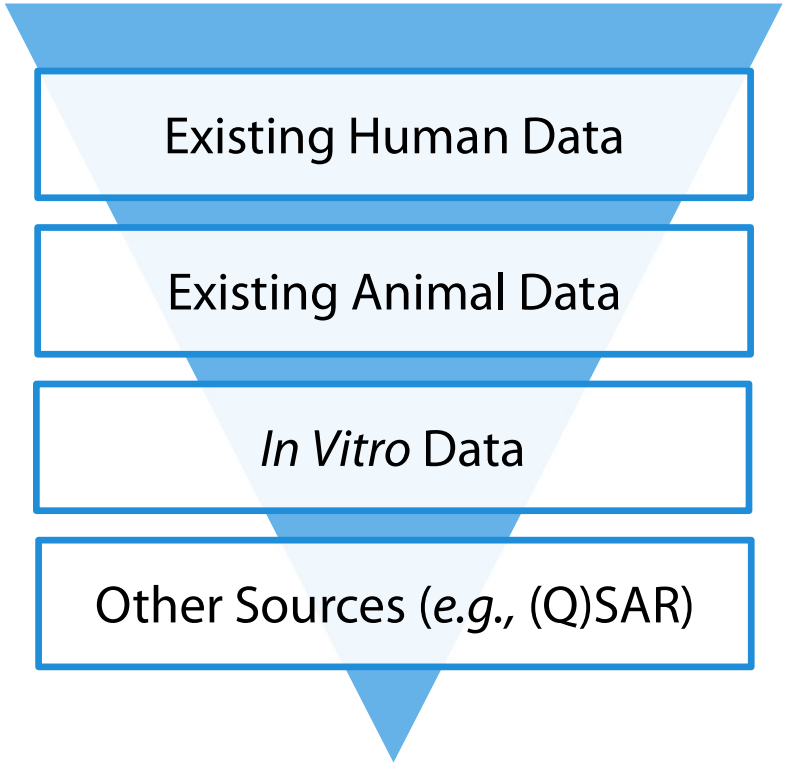
| Hazard Name | Hazard Class and Category | Hazard Code | Hazard Statement |
|---|---|---|---|
| Endocrine-Human Health Cat. 1 | ED HH 1 | EUH380 | May cause endocrine disruption in humans |
| Endocrine-Human Health Cat. 2 | ED HH 2 | EUH381 | Suspected of causing endocrine disruption in humans |
| Endocrine-Environment Cat. 1 | ED ENV 1 | EUH430 | May cause endocrine disruption in the environment |
| Endocrine-Environment Cat. 2 | ED ENV 2 | EUH431 | Suspected of causing endocrine disruption in the environment |
| Persistent, Bioaccumulative, & Toxic | PBT | EUH440 | Accumulates in the environment and living organisms, including in humans |
| Very Persistent, Very Bioaccumulative | vPvB | EUH441 | Strongly accumulates in the environment and living organisms, including in humans |
| Persistent, Mobile, & Toxic | PMT | EUH450 | Can cause long-lasting and diffuse contamination of water resources |
| Very Persistent, Very Mobile | vPvM | EUH451 | Can cause very long-lasting and diffuse contamination of water resource |

# New Hazard Classes

| Hazard Name | Hazard Class and Category | Hazard Code | Hazard Statement |
|---|---|---|---|
| Endocrine-Human Health Cat. 1 | ED HH 1 | EUH380 | May cause endocrine disruption in humans |
| Endocrine-Human Health Cat. 2 | ED HH 2 | EUH381 | Suspected of causing endocrine disruption in humans |
| Endocrine-Environment Cat. 1 | ED ENV 1 | EUH430 | May cause endocrine disruption in the environment |
| Endocrine-Environment Cat. 2 | ED ENV 2 | EUH431 | Suspected of causing endocrine disruption in the environment |
| Persistent, Bioaccumulative, & Toxic | PBT | EUH440 | Accumulates in the environment and living organisms, including in humans |
| Very Persistent, Very Bioaccumulative | vPvB | EUH441 | Strongly accumulates in the environment and living organisms, including in humans |
| Persistent, Mobile, & Toxic | PMT | EUH450 | Can cause long-lasting and diffuse contamination of water resources |
| Very Persistent, Very Mobile | vPvM | EUH451 | Can cause very long-lasting and diffuse contamination of water resource |

# Recent Regulatory Developments in the EU: New ED Hazard Classes Under CLP

## Weight-of-Evidence Approach Using Existing Data




**Endocrine Activity**

+

**Adverse Effect**

+

**Biologically Plausible Link**

=

**Endocrine Disruptor**

*"Interaction with the endocrine system that may result in a response of that system, or target organs or target tissues, and that confers on a substance or the mixture the potential to alter one or more functions of the endocrine system"*

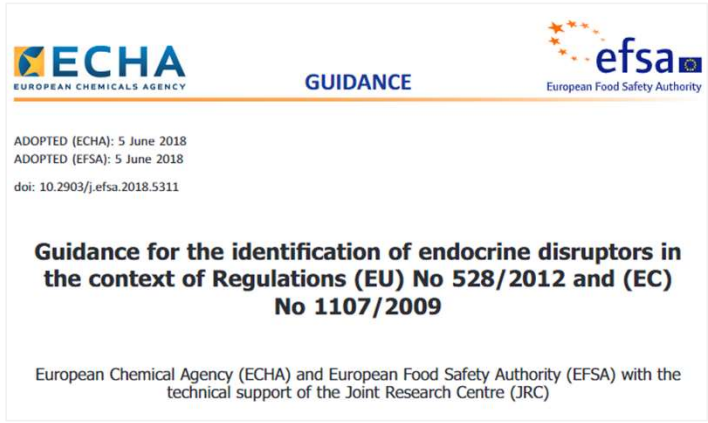
*"Change in morphology, physiology, growth, development, reproduction or lifespan of an organism, system, population or subpopulation that results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress or an increase in susceptibility to other influences"*

*"Correlation between an endocrine activity and an adverse effect, based on biological processes, where the correlation is consistent with existing scientific knowledge"*

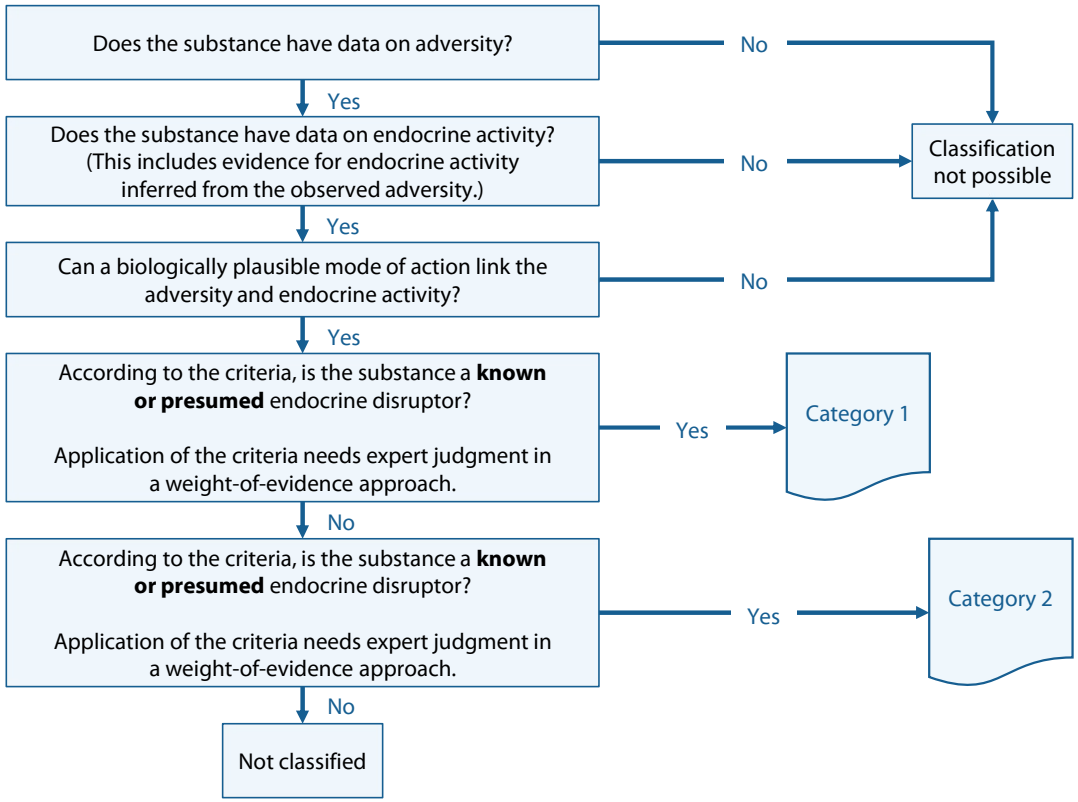
Source: ECHA (2017). Microsoft Word - CLP_Guidance_ED_revised_with_headings.docx (europa.eu).

# Evaluating Your Ingredient Portfolio

- Impact to regulated community could be substantial
- Evaluate portfolio by relying on:
  - Draft CLP guidance
  - EFSA/ECHA guidance for BP and PPP (focus on EATS)
  - OECD Conceptual Framework for EDs
- Use a structured, stepwise process:
  - Assess vulnerabilities
  - Identify data gaps
  - Identify priorities
  - Develop action plan/resources

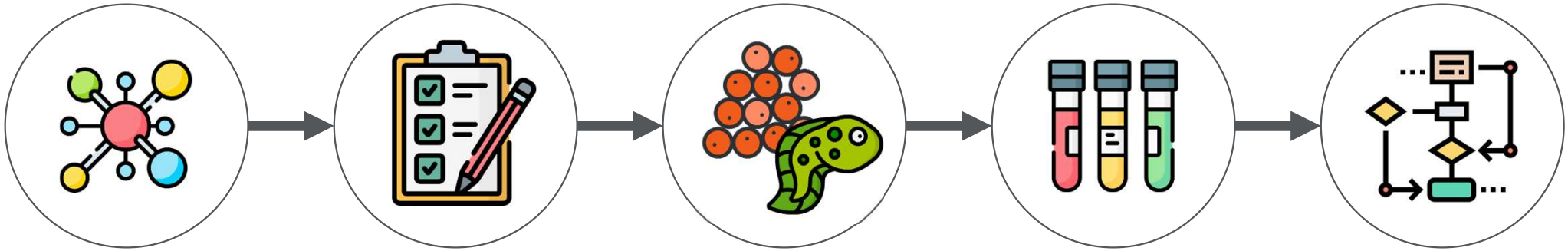



# The Process Flow Chart




Source: ECHA (2017). Microsoft Word - CLP_Guidance_ED_revised_with_headings.docx (europa.eu).

# Evaluating Your Ingredient Portfolio for ED-Stepwise Process



**STEP 1**

Assign Unique Substance Identifier

**STEP 2**

Compare to Existing ED Lists

**STEP 3**

Evaluate Endocrine Adversity

**STEP 4**

Evaluate Endocrine Activity

**STEP 5**

Evaluate Biological Plausibility

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# Assign Unique Substance Identifiers

STEP 1  STEP 2  STEP 3  STEP 4  STEP 5 

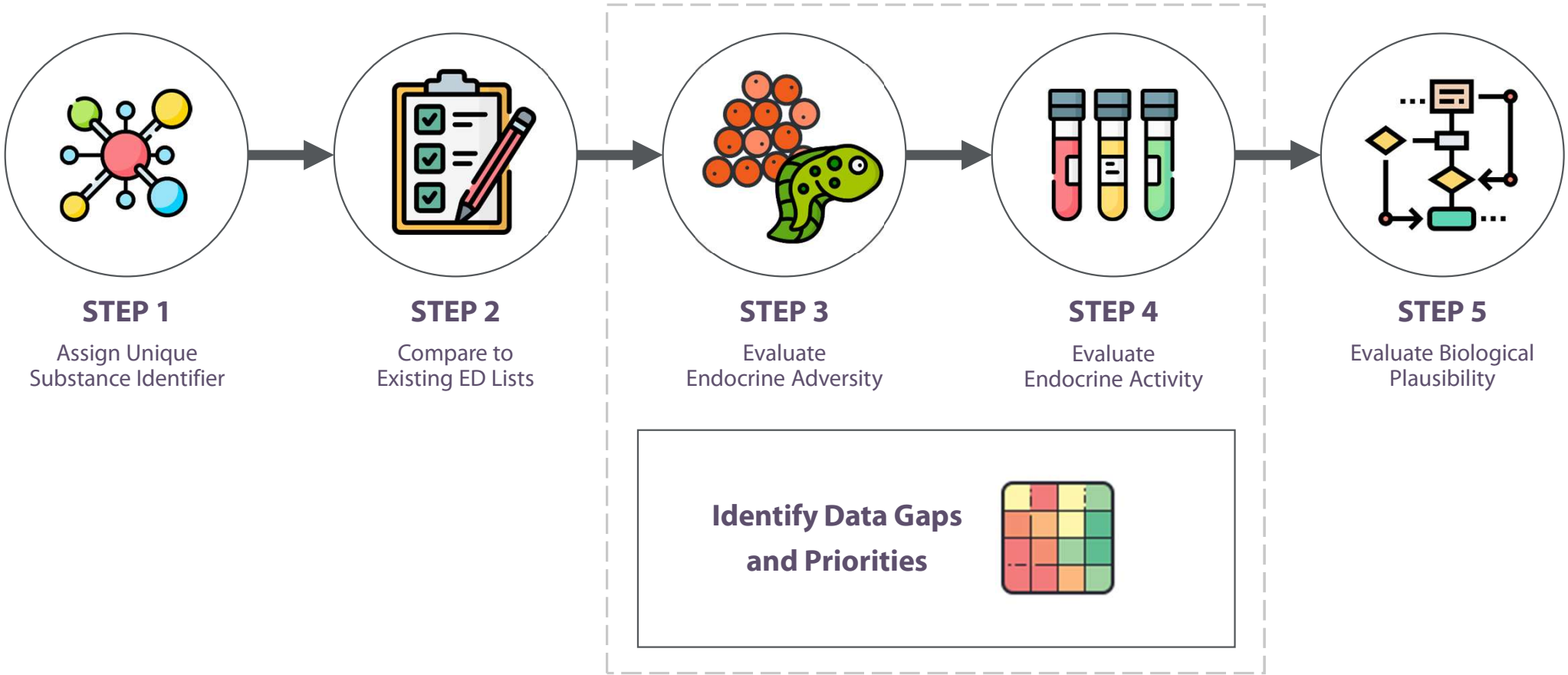
| Trans-decalin | |
|---|---|
| Chemical Abstracts Service (CAS) Number | 493-01-6 |
| European Inventory of Existing Commercial Substances (EINECS) Number | 202-046-9 |
| International Chemical Identifier (InChiKey) | NNBZCPXTIHJBJL-UHFFFAOYSA-N |
| Simplified Molecular-Input Line-Entry System (SMILES) | C1CCCC2CCCCC12 |
| International Union of Pure and Applied Chemistry (IUPAC) | 1,2,3,4,4a,5,6,7,8,8a-decahydronaphthalene |
| Distributed Structure-Searchable Toxicity Substance Identifier (DTXSID) | DTXSID00873337 |

# Compare to Existing ED Lists

STEP 1  **STEP 2**  STEP 3  STEP 4  STEP 5 

- Authoritative and screening lists
  - ECHA's ED Assessment list (https://echa.europa.eu/ed-assessment)
  - Candidate list of SVHC for ED under REACH (https://www.echa.europa.eu/candidate-list-table)
  - ED lists (https://edlists.org/the-ed-lists)
  - UNEP lists (https://wedocs.unep.org/bitstream/handle/20.500.11822/25633/EDC_report1.pdf?sequence=1&isAllowed=y)
  - ChemSec SIN list (https://sinlist.chemsec.org/endocrine-disruptors/)
  - Japan SPEED '98 list (https://www.env.go.jp/en/chemi/ed/speed98/sp98t3.html)
  - TEDX list (https://endocrinedisruption.org/interactive-tools/tedx-list-of-potential-endocrine-disruptors/search-the-tedx-list)
- Certain EU ED assessments are adequate to classify under CLP
  - ED under BP/PPP procedures → Assigned ED HH 1 and ED ENV 1 in CLP
  - SVHC for ED under REACH → Assigned ED HH 1 and ED ENV 1 in CLP

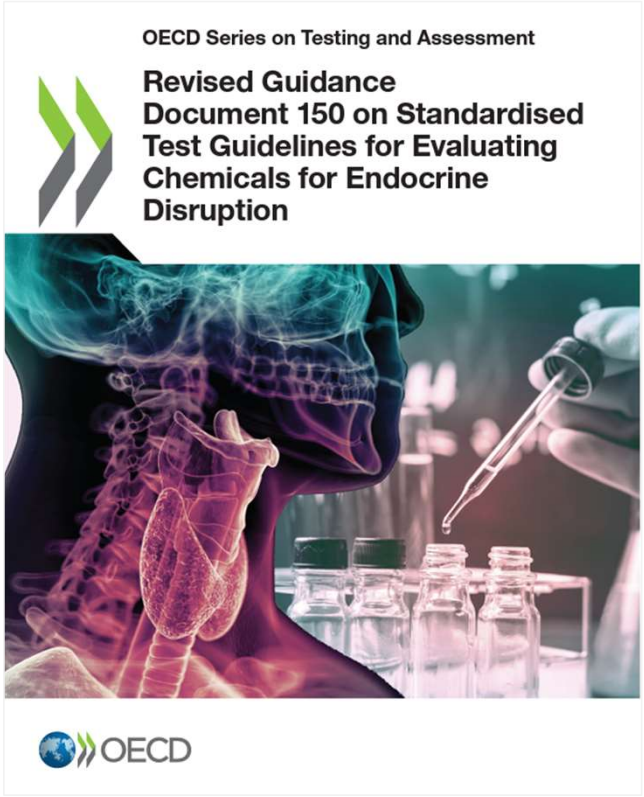
# Evaluating Your Ingredient Portfolio for ED



# Evaluate Endocrine **Adversity** (Human Health)

STEP 1 | STEP 2 | **STEP 3** | STEP 4 | STEP 5

| OECD Level | Test/Data Type | Example Endpoints |
|---|---|---|
| **Level 3** | • Uterotrophic bioassay in rodents<br>• Hershberger bioassay (H assay) | • Possible liver weight increase (in combination with other thyroid-related endpoints)<br>• Changes in serum T4 and T3 |
| **Level 4** | • 28/90-day repeated dose study<br>• Reproduction/developmental toxicity screening test<br>• Combined chronic toxicity and carcinogenicity studies | • Changes in sperm parameters: sperm numbers, sperm motility, sperm morphology<br>• Histopathologic changes in the above organs and in mammary glands<br>• Serum T4, T3 decreased, TSH increased; histopathologic changes in thyroid gland |
| **Level 5** | • Extended one-generation reproductive toxicity study<br>• Two-generation reproduction toxicity study | • Litter size, sex ratio (F1, F2), litter/pup weight, pup survival index, abnormalities in pup development<br>• Anogenital distance |



# Evaluate Endocrine **Adversity** (Human Health)

STEP 1 | STEP 2 | **STEP 3** | STEP 4 | STEP 5

DRAFT Evaluation Notes: Adverse effects on EATS-mediated parameters alone sufficient for classification (no endocrine activity or mechanistic evaluation required)

| EXAMPLE: Indicative of EATS-mediated Effects | EXAMPLE: Sensitive to, But Not Diagnostic of, EATS-mediated Effects |
|---|---|
| Age at vaginal opening | Adrenal weight |
| Anogenital distance | Brain histopathology |
| Epididymis weight | Litter size |
| Estrous cyclicity | Number of ovarian follicles |
| Sperm motility | Motor activity |
| Thyroid weight | Pre- and post-implantation loss |
| Liver weight* | Pituitary weight |
| Uterus weight | Pup development |

*With thyroid effects.

# Evaluate Endocrine **Adversity** (Human Health)

STEP 1 | STEP 2 | **STEP 3** | STEP 4 | STEP 5

- DRAFT Evaluation Notes:
  - If reproductive or repeated dose hazard, can still be classified for ED
    - ED effects can be classified even if some maternal toxicity
  - No dose threshold, so can be classified for ED, even if not classified for specific target organ toxicity (repeat exposure) (STOT RE)
    - For example, even if thyroid was not sensitive endpoint
  - Reversibility not considered
  - Non-specific/secondary effects do not need to be classified
  - Data from similar chemicals relevant
  - Structured guidance for EATS, but non-EATS still needs to be evaluated

# Evaluate Endocrine **Activity** (Human Health and Environment)

STEP 1  STEP 2  STEP 3  **STEP 4**  STEP 5 

## Activity Assessment

| OECD Level | Test/ Data Type | Example information |
|---|---|---|
| **Level 1** | Existing data and non-test information | • Physical and chemical properties<br>• All available (eco)toxicological data from (non-) standardized tests<br>• Read-across, chemical categories, QSARs and *in silico* prediction<br>• Scientific literature |
| **Level 2** | *In vitro* mechanistic assays (mammalian and non-mammalian methods) | • ER/AR binding and transactivation assay<br>• Steroidogenesis *in vitro*<br>• Aromatase assay<br>• Thyroid disruption assays<br>• High-throughput screens |

## Primary Data Sources

- US EPA's ToxCast™
- US EPA's Collaborative Estrogen Receptor Activity Prediction Project (CERAPP)
- US EPA's Collaborative Modeling Project for Androgen Receptor Activity (CoMPARA)
- QSAR DataBank (QsarDB)
- Danish (Quantitative) Structure-Activity Relationship [(Q)SAR] Database

# Evaluate Endocrine **Activity** (Human Health and Environment)

STEP 1 | STEP 2 | STEP 3 | **STEP 4** | STEP 5

## Example ToxCast™ Output

| Chemical | Agonism Score | Translation |
|---|---|---|
| 1,3-Dimethylbenzene | 0 | Inactive |
| Dibenz(a,h)anthracene | 0 | Inactive |
| 4-Octylphenol | 0.118 | Agonist |
| Dibenzofuran | 0.00597 | Inconclusive |
| Fluorene | 0 | Inactive |
| Acenaphthylene | 0 | Inactive |
| Anthracene | 0.0112 | Inconclusive |
| Benzo(b)fluoranthene | 0.012 | Inconclusive |

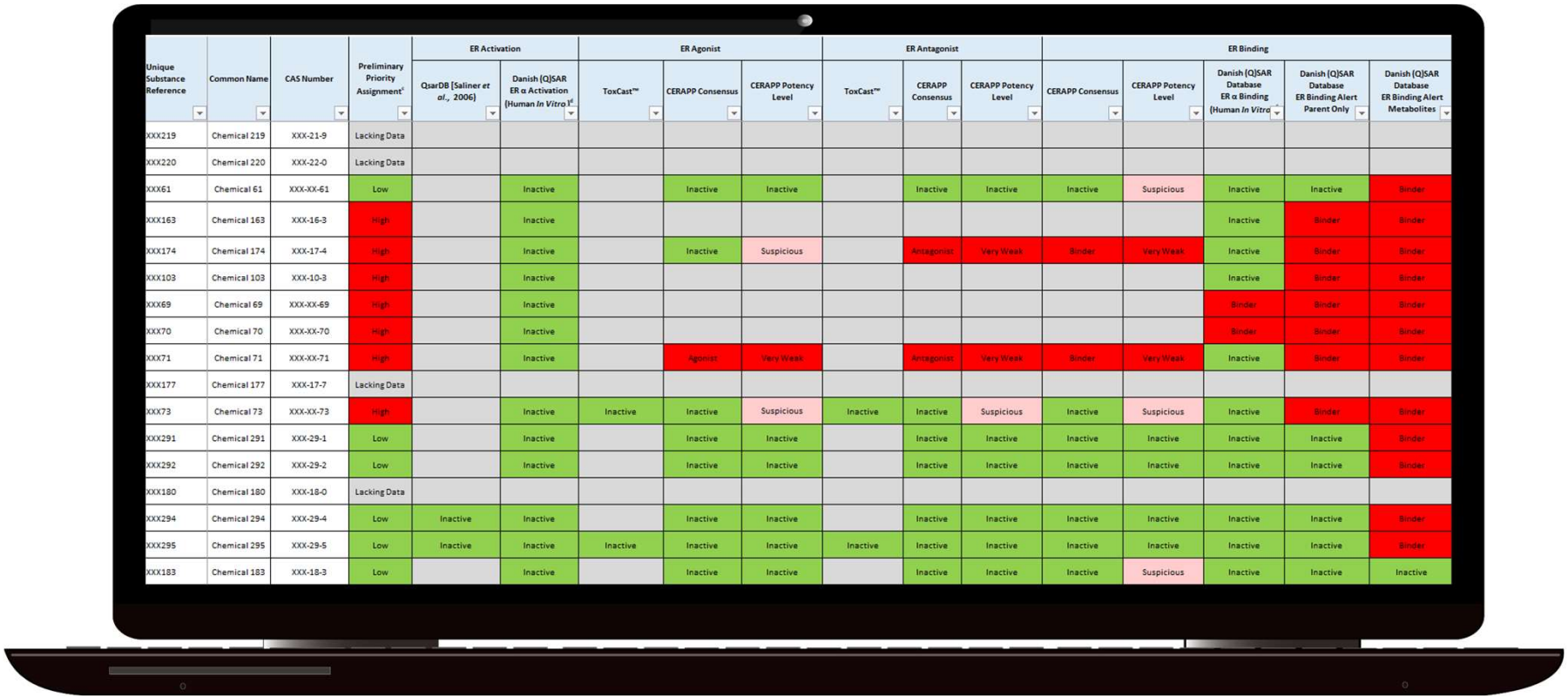
**Agonist or Antagonist:** Values ≥0.1; **Inconclusive:** Values 0.001-0.1; and **Inactive:** AUC values <0.001.

## Example: Estrogen Activity

- ER Agonism = CERAPP and ToxCast™
- ER Antagonism = CERAPP and ToxCast™
- ER Binding = CERAPP and Danish (Q)SAR
- Binding (human *in vitro*), alerts for parent and metabolites
- ER Activation = ER α activation
- (Human *in vitro*) in QsarDB and Danish (Q)SAR

# Identify Data Gaps and Priorities Based on Endocrine **Activity** (Heat Map Approach)

STEP 1 | STEP 2 | STEP 3 | **STEP 4** | STEP 5



| Unique Substance Reference | Common Name | CAS Number | Preliminary Priority Assignment[c] | ER Activation | | ER Agonist | | | ER Antagonist | | | ER Binding | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | QsarDB (Saliner *et al.*, 2006) | Danish (Q)SAR ER α Activation (Human *In Vitro*)[d] | ToxCast™ | CERAPP Consensus | CERAPP Potency Level | ToxCast™ | CERAPP Consensus | CERAPP Potency Level | CERAPP Consensus | CERAPP Potency Level | Danish (Q)SAR Database ER α Binding (Human *In Vitro* | Danish (Q)SAR Database ER Binding Alert Parent Only | Danish (Q)SAR Database ER Binding Alert Metabolites |
| XXX219 | Chemical 219 | XXX-21-9 | Lacking Data | | | | | | | | | | | | | |
| XXX220 | Chemical 220 | XXX-22-0 | Lacking Data | | | | | | | | | | | | | |
| XXX61 | Chemical 61 | XXX-XX-61 | Low | | Inactive | | Inactive | Inactive | | Inactive | Inactive | Inactive | Suspicious | Inactive | Inactive | Binder |
| XXX163 | Chemical 163 | XXX-16-3 | High | | Inactive | | | | | | | | | Inactive | Binder | Binder |
| XXX174 | Chemical 174 | XXX-17-4 | High | | Inactive | | Inactive | Suspicious | | Antagonist | Very Weak | Binder | Very Weak | Inactive | Binder | Binder |
| XXX103 | Chemical 103 | XXX-10-3 | High | | Inactive | | | | | | | | | Inactive | Binder | Binder |
| XXX69 | Chemical 69 | XXX-XX-69 | High | | Inactive | | | | | | | | | Binder | Binder | Binder |
| XXX70 | Chemical 70 | XXX-XX-70 | High | | Inactive | | | | | | | | | Binder | Binder | Binder |
| XXX71 | Chemical 71 | XXX-XX-71 | High | | Inactive | | Agonist | Very Weak | | Antagonist | Very Weak | Binder | Very Weak | Inactive | Binder | Binder |
| XXX177 | Chemical 177 | XXX-17-7 | Lacking Data | | | | | | | | | | | | | |
| XXX73 | Chemical 73 | XXX-XX-73 | High | | Inactive | Inactive | Inactive | Suspicious | Inactive | Inactive | Suspicious | Inactive | Suspicious | Inactive | Binder | Binder |
| XXX291 | Chemical 291 | XXX-29-1 | Low | | Inactive | | Inactive | Inactive | | Inactive | Inactive | Inactive | Inactive | Inactive | Inactive | Binder |
| XXX292 | Chemical 292 | XXX-29-2 | Low | | Inactive | | Inactive | Inactive | | Inactive | Inactive | Inactive | Inactive | Inactive | Inactive | Binder |
| XXX180 | Chemical 180 | XXX-18-0 | Lacking Data | | | | | | | | | | | | | |
| XXX294 | Chemical 294 | XXX-29-4 | Low | Inactive | Inactive | | Inactive | Inactive | | Inactive | Inactive | Inactive | Inactive | Inactive | Inactive | Binder |
| XXX295 | Chemical 295 | XXX-29-5 | Low | Inactive | Inactive | Inactive | Inactive | Inactive | Inactive | Inactive | Inactive | Inactive | Inactive | Inactive | Inactive | Binder |
| XXX183 | Chemical 183 | XXX-18-3 | Low | | Inactive | | Inactive | Inactive | | Inactive | Inactive | Inactive | Suspicious | Inactive | Inactive | Inactive |

# Evaluate Endocrine **Activity** (Human Health and Environment)

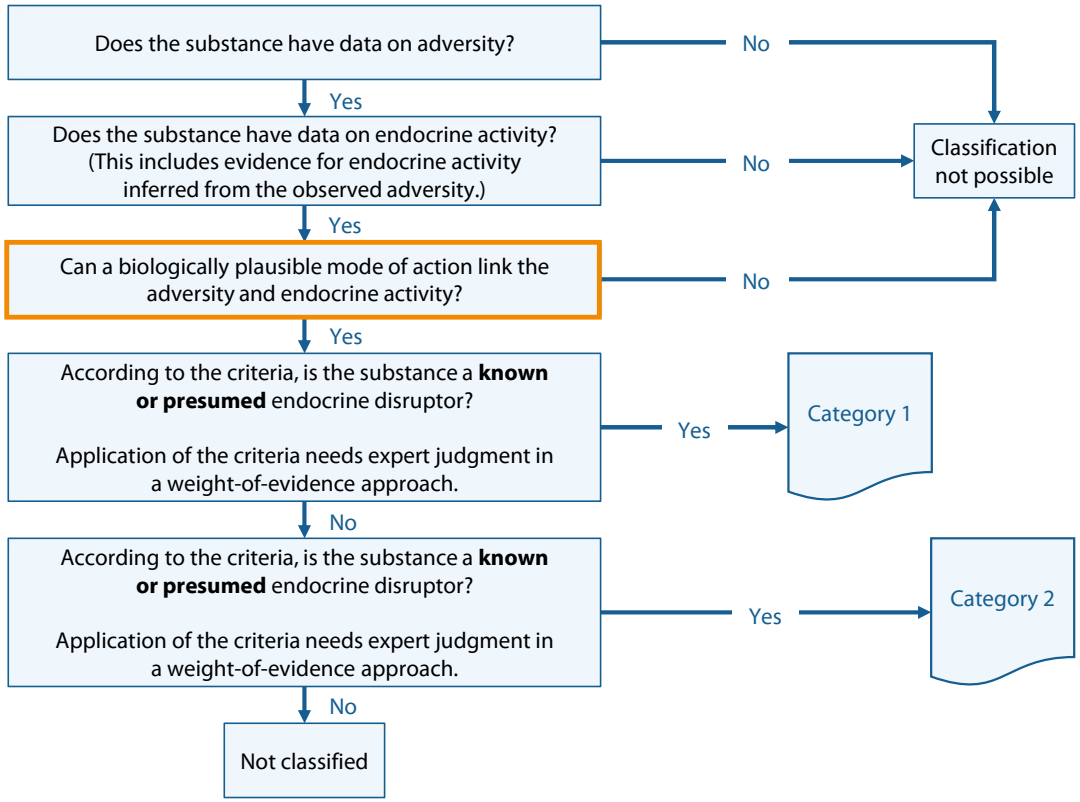
STEP 1 | STEP 2 | STEP 3 | **STEP 4** | STEP 5

## Scientific Literature

- Search strategies provided in EFSA (2018)
- Relatively easy to implement, but extracting relevant information is very resource intensive

# Prepare for Biological Plausibility Assessment (If Needed)

STEP 1 | STEP 2 | STEP 3 | STEP 4 | STEP 5




Identify Data Gaps and Priorities Based on Endocrine **Activity** and **Adversity**

Source: ECHA (2017). Microsoft Word - CLP_Guidance_ED_revised_with_headings.docx (europa.eu).

# Prepare for Biological Plausibility Assessment (If Needed)

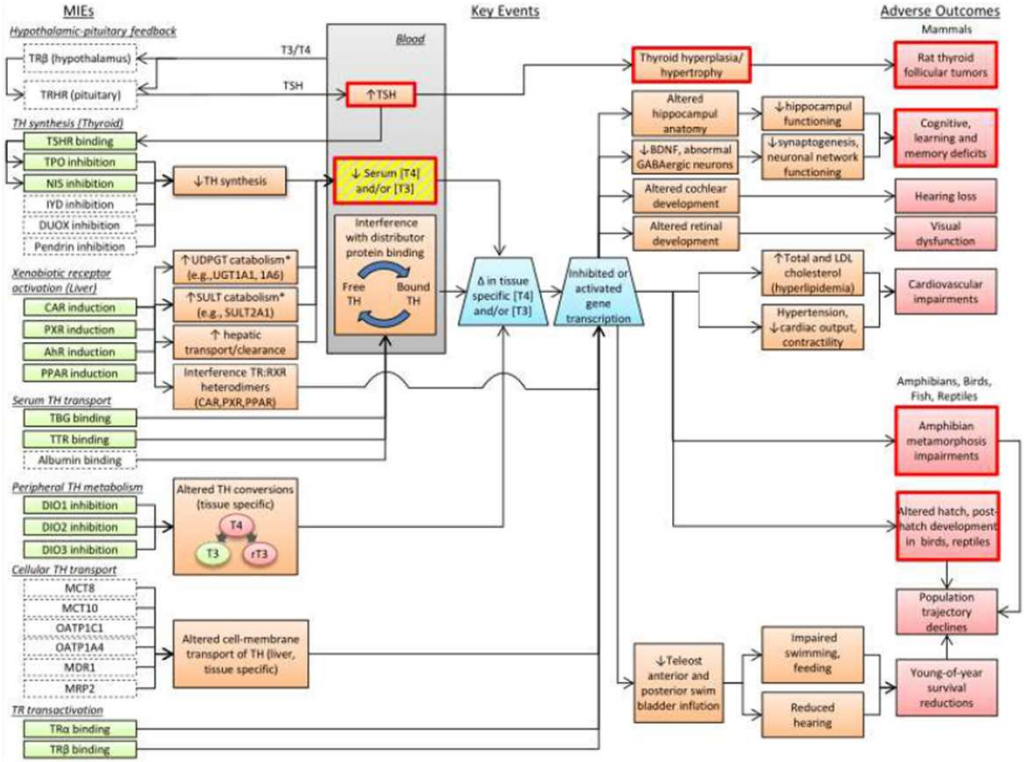
STEP 1  STEP 2  STEP 3  STEP 4  **STEP 5** 

## Prioritize for biological plausibility assessment?

| Chemical | Level 1 | Level 2 | Level 3 | Level 4 | Level 5 | Conduct Plausibility Assessment |
|---|---|---|---|---|---|---|
| 1 | | ToxCast: Active | | 90-day study: No adverse effects | Two-gen study: No adverse effects | No |
| 2 | Peer-reviewed literature: Mixed results | ToxCast: Active | | 90-day study: Adverse effects | Two-gen study: Adverse effects (sperm motility) | No |
| 3 | Peer-reviewed literature: Mixed results | ToxCast: Inactive | | 90-day study: Adverse effects | Two-gen study: Adverse effects (sperm motility) | Yes? |
| 4 | Peer-reviewed literature: Mixed results | ToxCast: Active | | 90-day study: Adverse effects | Two-gen study: Adverse effects (litter size) | Yes |
| 5 | | ToxCast: Inactive | | | | No |

# Evaluate Biological Plausibility

STEP 1 | STEP 2 | STEP 3 | STEP 4 | STEP 5




Source: ECHA (2017). Microsoft Word - CLP_Guidance_ED_revised_with_headings.docx (europa.eu).

- Evaluate **biological plausibility** only for key chemicals
  - Can be a significant effort
  - Requires expert judgment
  - Relies on mode-of-action and weight-of-evidence approach
  - Analogy, essentiality, consistency, specificity, temporal concordance



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# Evaluate Biological Plausibility: EDHH1 or EDHH2

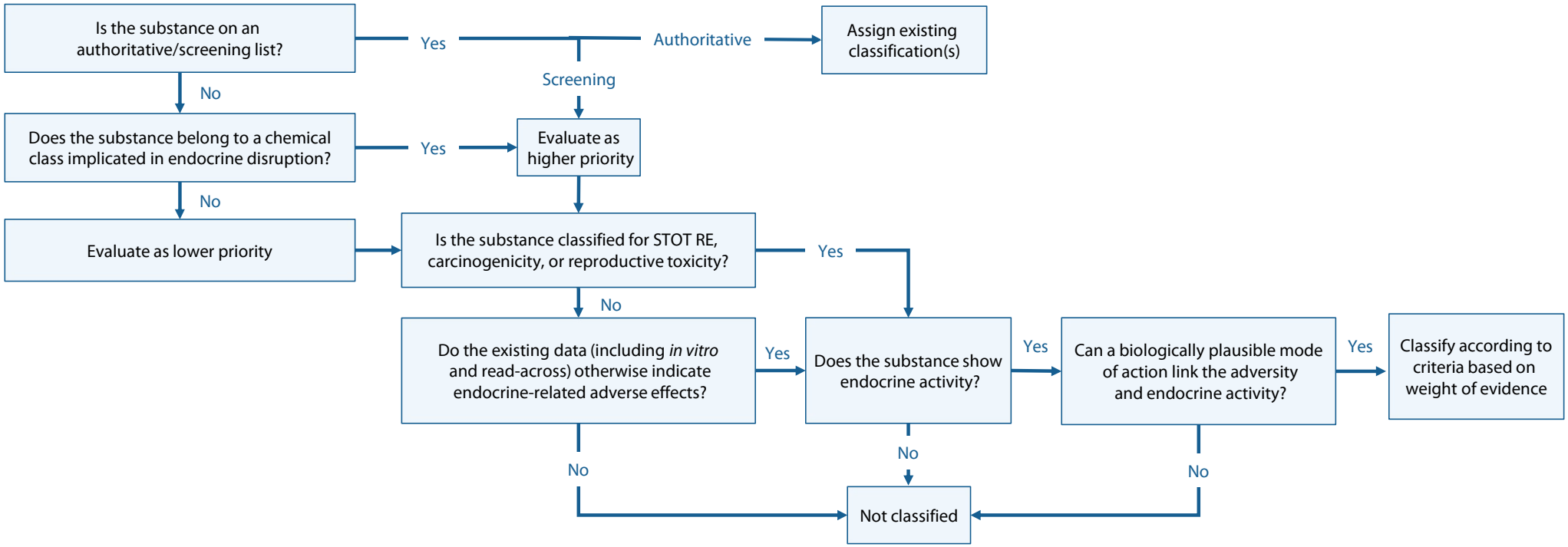
STEP 1  STEP 2  STEP 3  STEP 4  **STEP 5** 

Classification Considerations for Endocrine Disruptor for Human Health (ED HH)

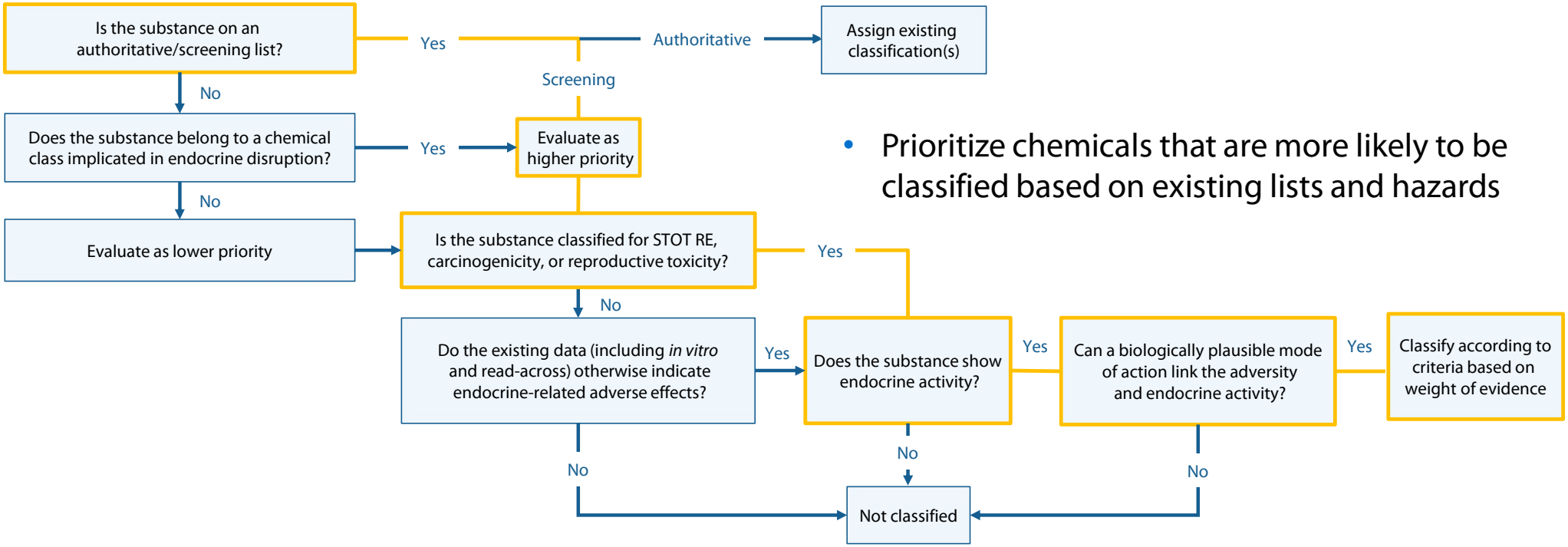
<table>
<tr><th>Category 1</th><th>Category 2 - Suspected</th><th>No Classification</th></tr>
<tr><td>Known or presumed</td><td>Suspected</td><td>Not classified</td></tr>
<tr><td colspan="2">Evidence for endocrine activity, adverse effect, and biologically plausible link</td><td>One or more of the 3 elements (adversity, activity, biological link) are missing</td></tr>
<tr><td>Human relevance cannot be excluded</td><td>Serious doubt about the human relevance of observed adverse effects</td><td>Convincing evidence that human relevance can be excluded</td></tr>
<tr><td rowspan="2">Clear evidence for adversity (e.g., pattern of effects, consistency across lines of evidence), which cannot be attributed to general toxicity</td><td>Evidence of endocrine activity and adversity are not sufficiently convincing for ED HH 1</td><td>Overall strength of evidence is not convincing enough for ED HH 2</td></tr>
<tr><td colspan="2">Slight changes or inconsistent findings across generations and/or studies:<br>- Histopathological effects that are mild or of low incidence<br>- Organ weight changes that are mild (e.g., 5%) or not statistically significant<br>- In vitro endocrine activity that is weak or only observed at cytotoxic concentrations</td></tr>
</table>

Source: ECHA (2024). Microsoft Word - CLP_Guidance_ED_revised_with_headings.docx (europa.eu).

# Prioritization Based on Endocrine **Activity** and **Adversity**

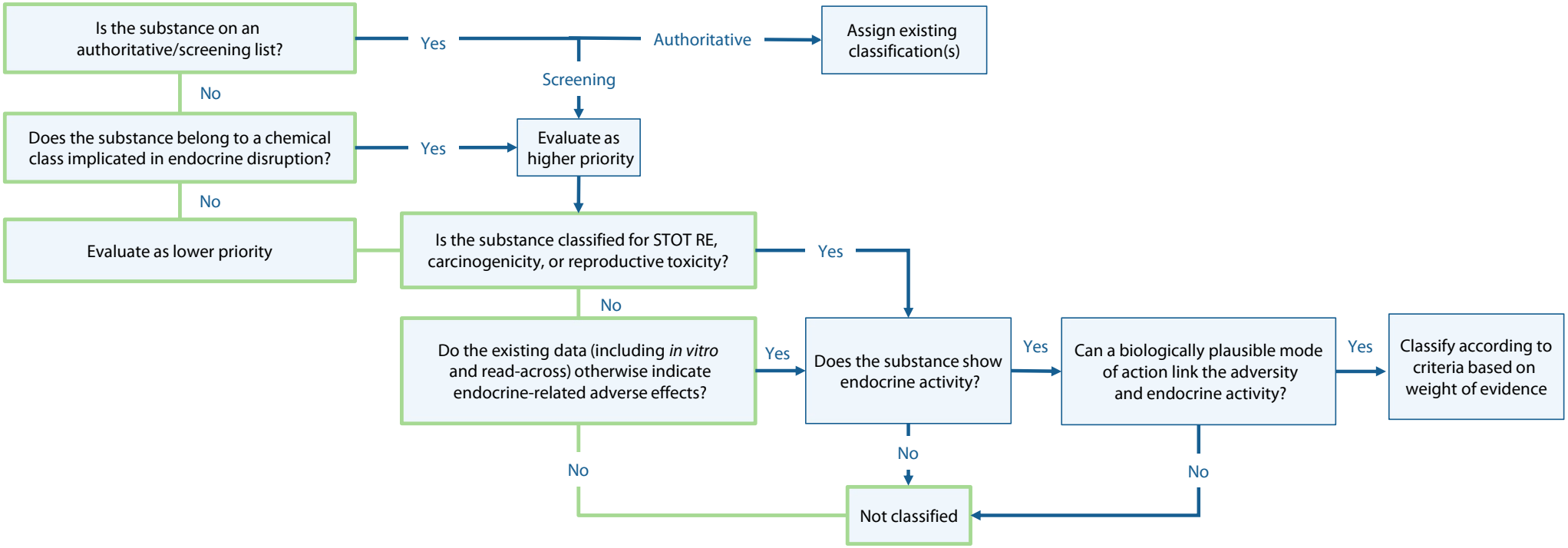


# Prioritization Based on Endocrine **Activity** and **Adversity**




- Prioritize chemicals that are more likely to be classified based on existing lists and hazards

# Prioritization Based on Endocrine **Activity** and **Adversity**



# Conclusions

- ED is now rapidly becoming a focus of several product/ingredient regulations
  - Proactively understanding portfolio vulnerabilities will minimize business disruptions
- Assessing ED across a portfolio is resource intensive
  - Prioritization using a structured process is advised
  - Plan for resources to resolve data gaps

# Thank You!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# Mobility Criterion (M)

- Defined as the affinity of a substance, once released to the environment, to spread over short or long distances and enter water bodies, including drinking water and groundwater.
- Indicated by the organic carbon water partition coefficient $K_{OC}$
- Type of information that can be considered for the assessment of M/vM properties:
  - Experimental data on adsorption deriving a $K_{OC}$ value (OECD TG 106, OECD TG 121, OECD TG 312, TLC)
  - Other experimental information deriving a $K_{OC}$ value (Field and lysimeter studies, OECD TG 22)
  - Data from estimation methods (e.g., QSARs) deriving a $K_{OC}$ value
  - Monitoring data
  - Other estimation approaches, including modelling not deriving a $K_{OC}$ value (Octanol-water distribution coefficient ($D_{OW}$), Leaching simulation modelling)
  - Aged sorption data
  - Specific considerations for ionizable substances

**Slide 31**

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**WTH1** Directly copied over from the guidance document
Wasfia T. Hoque, 11/29/2023

**TV58** ok- thanks!
Tim Verslycke, 11/29/2023

**WTH2** does not mention anything on QSAR
Wasfia T. Hoque, 11/29/2023

**TV57** QSARs are mentioned in third bullet or are you specifically referring to ionizable considerations?
Tim Verslycke, 11/29/2023