# Ecotoxicological Hazard and Risk Assessment of Endocrine Active Substances

Presented by Annegaaike Leopold

At ECHA workshop on Applications for Authorisation for Environmental Endocrine Disruptors – 22 August 2017

# The take home message for you:

48 global experts with equal representation from government, academia, industry and consultancies from 9 countries came together and **addressed the controversy at the heart of environmental safety assessment of endocrine disrupting substances (EDS)**

Disclaimer: The views or statements expressed in this presentation do not necessarily represent the views of the organisations to which the participants are affiliated

# SETAC Pellston Workshop® - Pensacola, FL, February 2016

48 participants

Academia: 27%
Government: 27%
Independent consultants: 25%
Industry: 21%

North America (48%)
Europe (42%)
Japan (6%)
Australia (4%)

# What is SETAC?

**The Society of Environmental Toxicology and Chemistry (SETAC)** is a not-for-profit, global professional organization comprised of some 6,000 individual members and institutions from academia, business and government.

Founded in 1979 the SETAC provides a forum for scientists, managers and other professionals to exchange information on, study, analyze and solve environmental problems through multidisciplinary approaches and provide environmental education.

**Science-based objectivity is a key founding principle.**

# Against the background of:

- Continuing scientific concern,  public debate, and media attention over some EDS that are, or have been, present in the environment at concentrations harmful to wildlife populations,
- Some jurisdictions initiating regulatory approaches but
- Lack of consensus about the most appropriate approaches:
  - Some scientists believe that EDS can be reliably assessed using the standard risk assessment paradigm,
  - others do not believe this is sufficiently precautionary and propose risk management on the basis of hazard alone

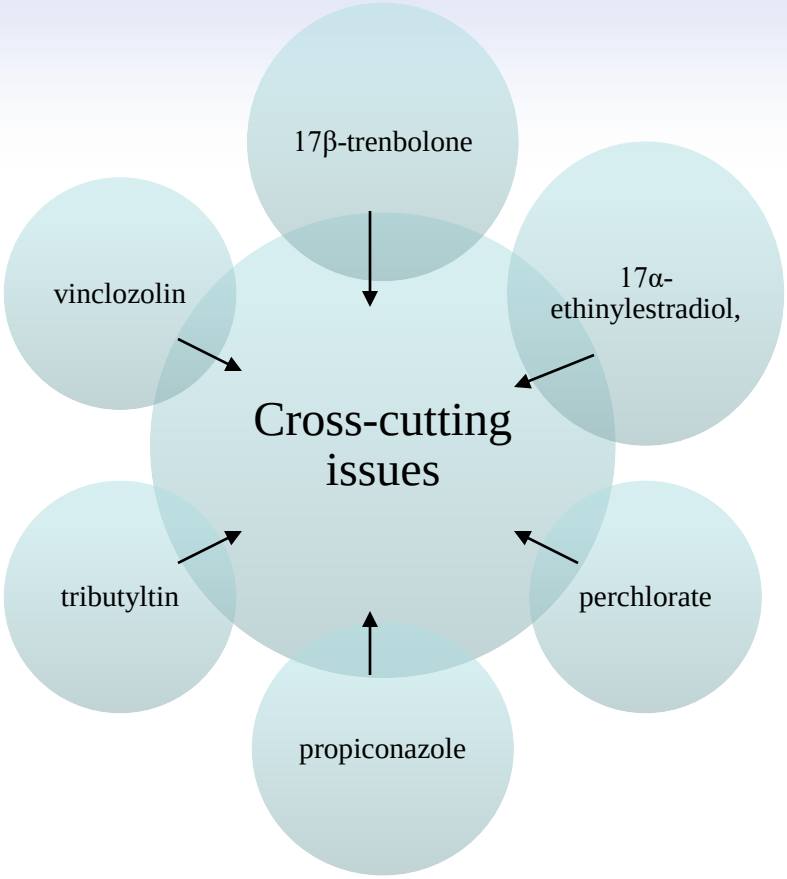
# The EHRA SETAC Pellston Workshop

**was held with the aim of:**

- trying to find scientific agreement on how best to assess the impact of endocrine active substances (EAS) on wildlife.
- developing guidance, assisting regulators and policy makers in their decision making, thereby contributing to speeding up the global progress in controlling these chemicals.
- identifying areas of uncertainty and knowledge gaps.

# Workshop Approach: Cross-cutting Issues from Six Case Studies

- Case studies summaries are included as SI to Matthiessen et al. IEAM (In Review )




- Endocrine active
- Data rich (several taxa; multiple levels of biological organisation; well studied)
- Covering range of ED pathways of concern
- NOT full safety evaluations but mechanism for identifying issues

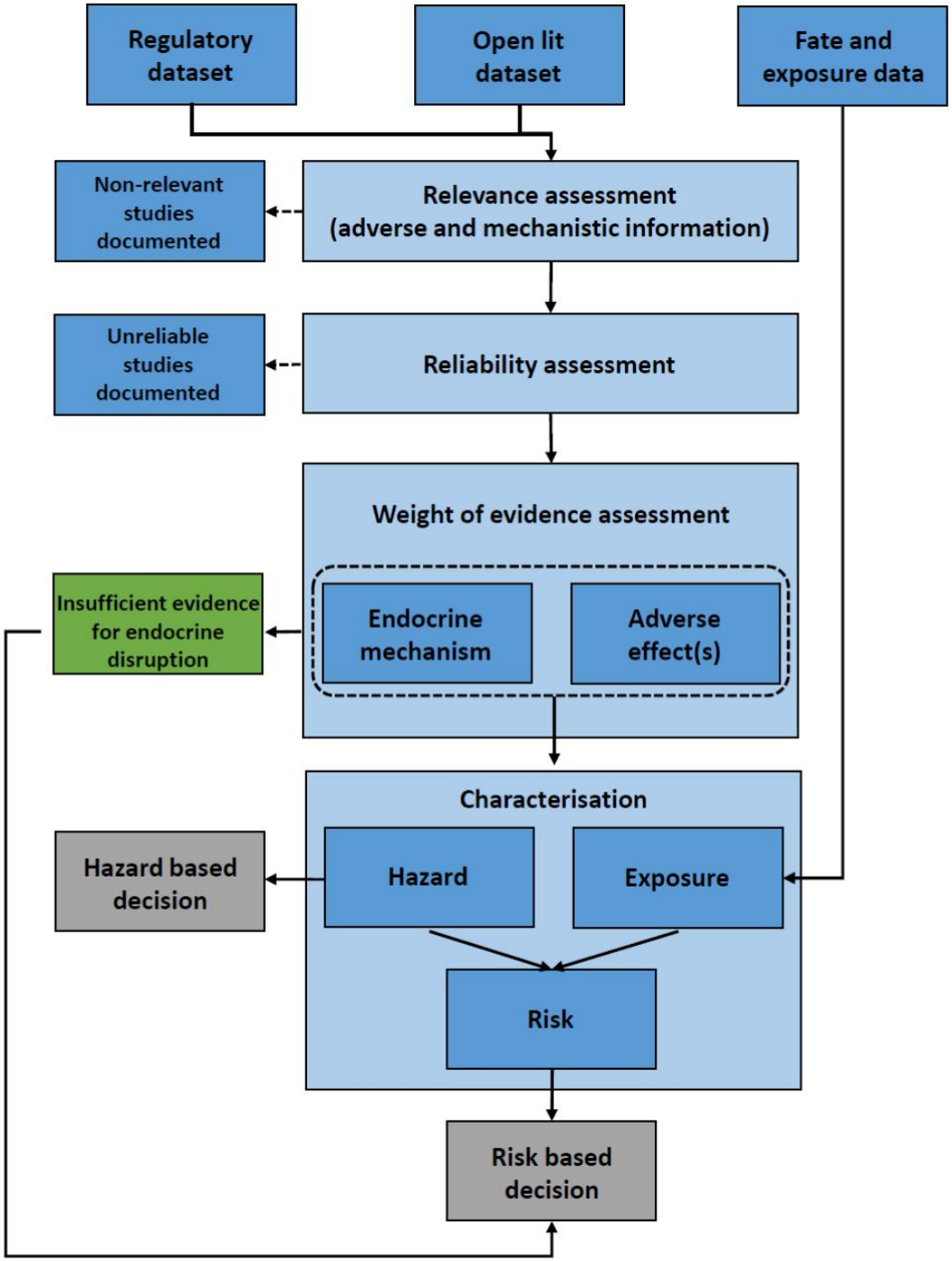
# Workshop Approach: Six Case Study chemicals:

- Focus for literature search based on use, exposure routes, MOA of chemicals; for example:
  - EE2: focus on aquatic environment (initially 15000 papers -> 1371 and from there focus on fish – vertebrate taxon with estrogen receptors.
  - Perchlorate: from 6945 ref. -> app. 180. Focus on repro/dev tox studies because development is sensitive lifestage for thyroid insuficciency. Focus on population level effects; confirm similar MOA across species.

# Workshop Approach:
# Six Case Study chemicals:

- TBT: From 965 regulatory reports + open and grey literature to 160. Fish (45 ref.), molluscs (55) and other taxonomic groups (60).
- Trenbolone: 805 ref. -> 155. Hazard and risk assessment focused on aquatic vertebrate species, because MOA of TRB is steroid hormone activity + major route of exposure is via animal waste run-off into aquatic systems.

## Workflow followed by Case Studies (Supplemental data in Matthiessen *et al* – 2017)



# Case study approach:

- **Quality evaluation of relevant data**: selected papers were:

- Toxicological Data Reliability Assessment Tool (ToxRTool - Schneider et al, 2009) and/or
- Klimisch criteria (Klimisch et al., 1997). Klimisch 1 and 2 used in the analysis, Klimisch score of 3 or 4 were not used.
- Histopath data addressed more specifically in parallel exercise and results incorporated in case study evaluations.

# Cross Cutting Issues: Uncertainties in Biological Responses

- For example:
  - effects manifest at a later life stage, following exposure during sensitive windows of life cycle, eg sex reversal in fish;
  - multigenerational effects reflected in design of extended one-generation designs for fish and mammals;
  - transgenerational effects
  - **non-monotonic dose relationships (NMDRs)/thresholds.**

# Threshold issues and NMDR's encountered in the case studies: *

- EE2:
  - NMDR for egg production (zebra fish, FHM).
  - NOECs for fish repro endpoints sometimes not determined due to effects seen at lowest conc tested.
  - But: sufficient fish full life cycle studies with LOEC/NOEC -> able to do risk assessment
- Perchlorate
  - NMDR in one study in stickleback T3 and T4 levels were affected at low concentrations but not at two higher concentrations across life stages (larval, juvenile and adult stages) No apical endpoints evaluated in this study -> relevance could not be determined.
  - NMDR in mammalian 2-gen: T4 but inconsistent across dose, gender and generation.
  - NMDR not seen population relevant endpoints -> no impact on risk assessment in case study.
- TBT: Inverted U shaped responses:
  - mainly involve gene expression studies
  - *In vivo:* eg effect on bw in fish and mammals -> different modes of action operating at different does.
- Trenbolone –
  - U-shaped dr relationship for plasma T, E2 and VTG conc in female FHM exp to TRB for 21 days. Conc > PEC conc -> little significance.
  - NMDRs seen for steroid synthesis/ concentration and or HPG gene expression I in FHM . Biological basis given by Ankley & Villeneuve, 2015 relates to compensatory responses during exposure and early recovery after removal of chemical stressor.

# Conclusion Regarding Threshold Issues and NMDR's encountered in the case studies: *

***Are the responses significant, plausible, repeatable, across species; endocrine mediated, multiple MOA's, adaptive process, exposure artefacts?***

- NMDRs seen in case studies were not considered to impede an environmental risk assessment.
- In nearly all cases threshold determination was straightforward. In other words: studies that scored a Klimisch rating of 1 or 2 described experiments with apical endpoints and exposure concentration ranges that allowed thresholds to be resolved for adverse population-relevant effects. In all of these case studies, for apical population-relevant endpoints we were able to determine clear thresholds for endocrine mediated adverse effects for the purpose of risk assessment.

# Uncertainties in biological responses eg NMDRs

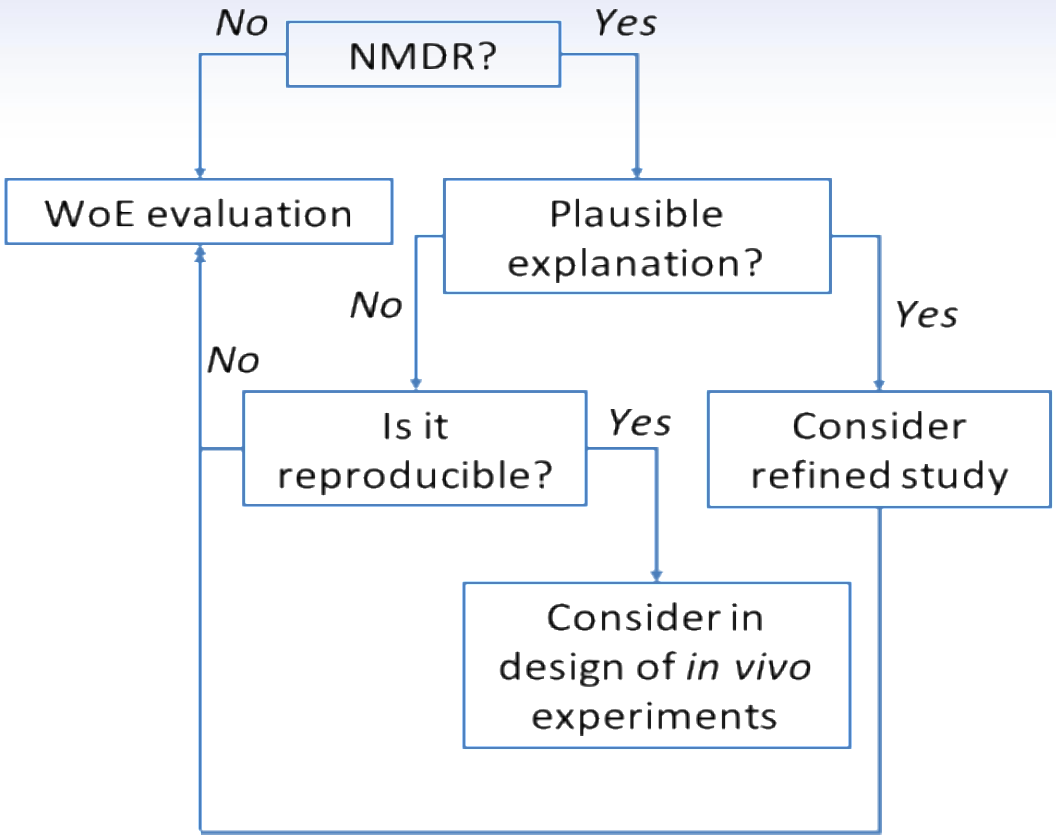



Fig 1A (from Parrott et al, 2017): Flowchart for evaluation of NMDR's for mechanistic endpoints (*in vitro* and biomarkers

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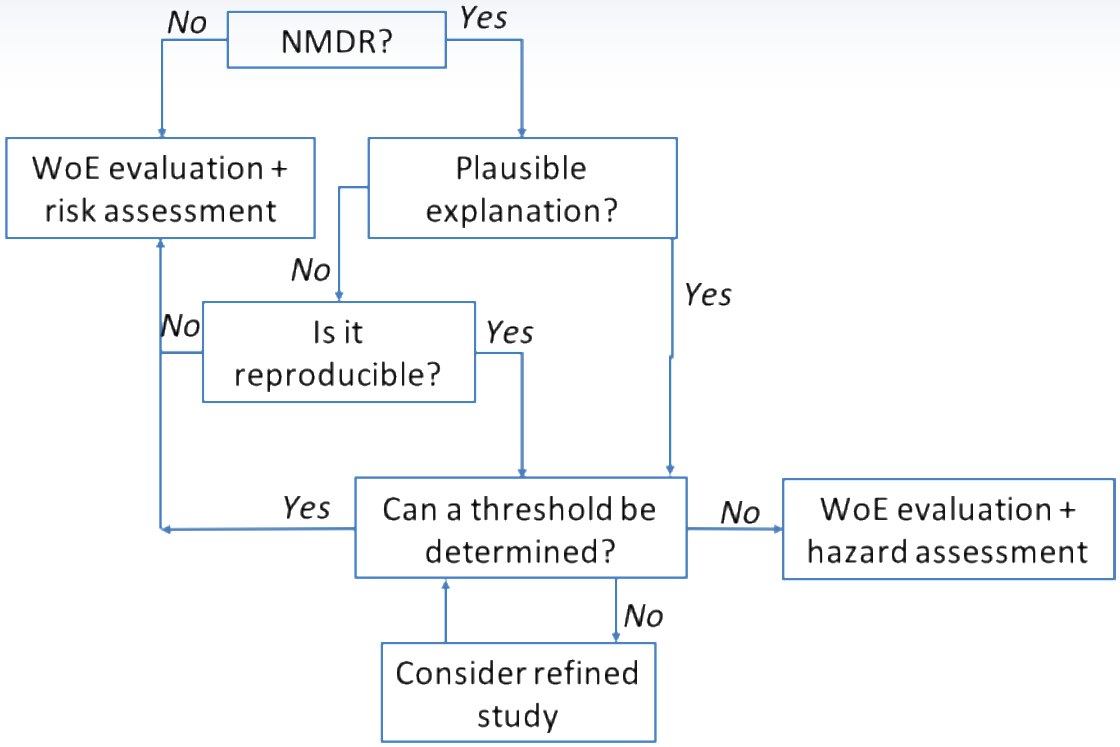



Fig 1B (from Parrott et al, 2017): Flowchart for evaluation of NMDR's for *in vivo* apical and/or population level endpoints

# Cross Cutting Issues: Improved methods for assessment of EAS/EDS *

Opportunities to improve approaches to, and guidance for existing test methods, and reduce uncertainty eg:

- *in vitro* high throughput screening to prioritize chemicals for testing and provide insights as to the most appropriate assay(s) for characterizing hazard and risk.
- adding endpoints for elucidating connections between mechanistic effects and adverse outcomes,
- identifying potentially sensitive taxa for which test methods currently do not exist
- addressing key endocrine pathways of possible concern in addition to those associated with estrogen, androgen and thyroid (EAT) signalling.

*** Coady et al, 2017**

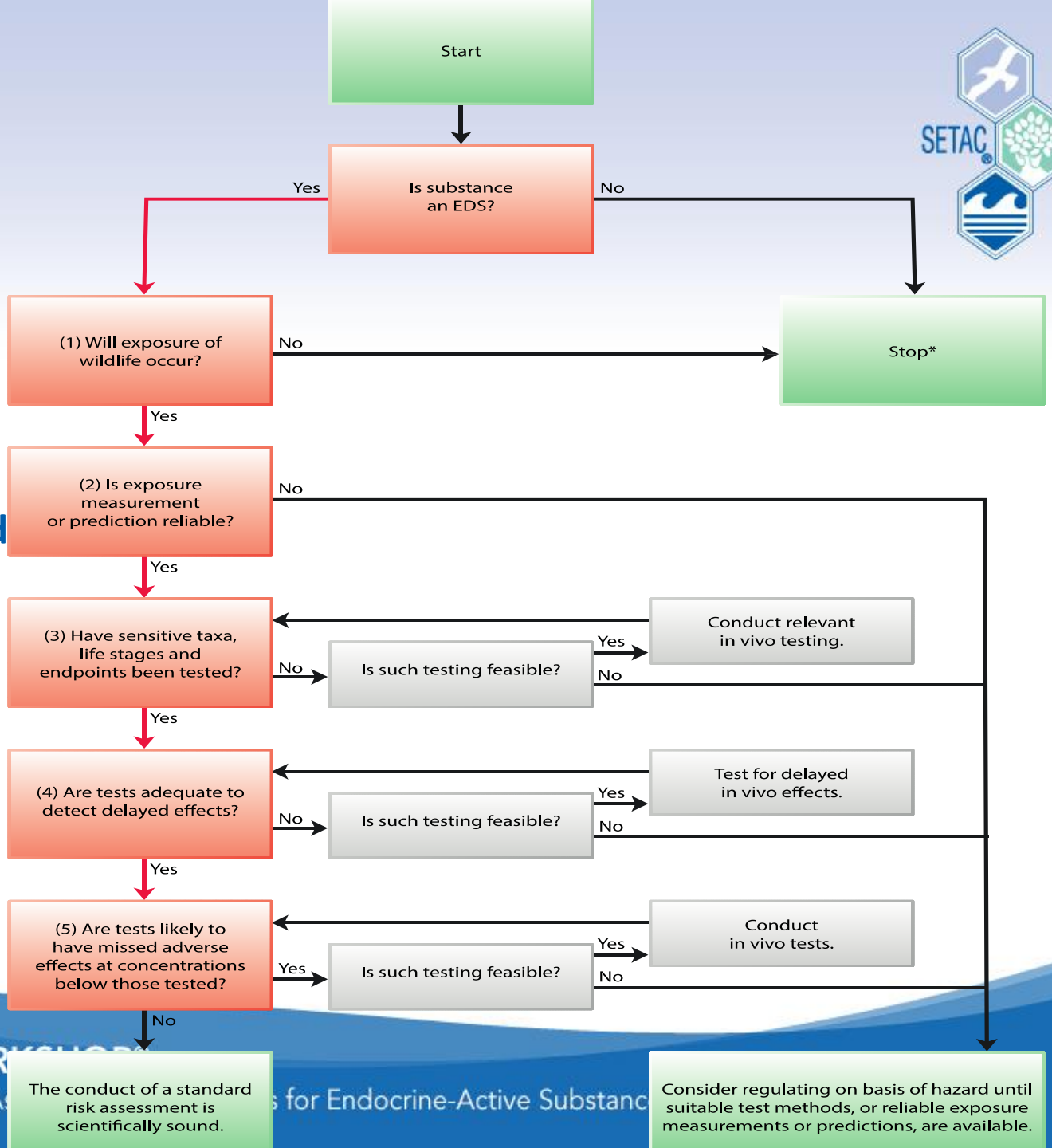
# Cross Cutting Issues: Identifying Population-relevant Endpoints in the Evaluation of EAS *

- There are a few examples of population level impacts from EAS exposure (eg gastropods and TBT; Fish and EE2)
- Greater understanding of relationship between mechanistic (in vitro, non-apical) endpoints and adverse population-level effects is needed.
- Consider severity and prevalence of response
- Use newer endpoints (eg behaviour) from current / newly designed studies.
- Development of adverse outcome pathways (AOPs) and population modeling will increase this understanding.
- Recovery process should be considered when evaluating population-level effects.

*** Marty et al, 2017.**

# Proposed Decision-making Strategy to Support Endocrine Disrupter Ecotoxicological Hazard and Risk Assessment

(Matthiessen *et al*, 2017)



# Main Conclusions of the Workshop

Provided:

- environmental exposure,
- effects on relevant taxa and life-stages,
- delayed effects,
- dose- and concentration-response relationships

are adequately characterized, then conducting environmental risk assessment of endocrine disrupting substances is scientifically sound; expected to be the case for most EAS;

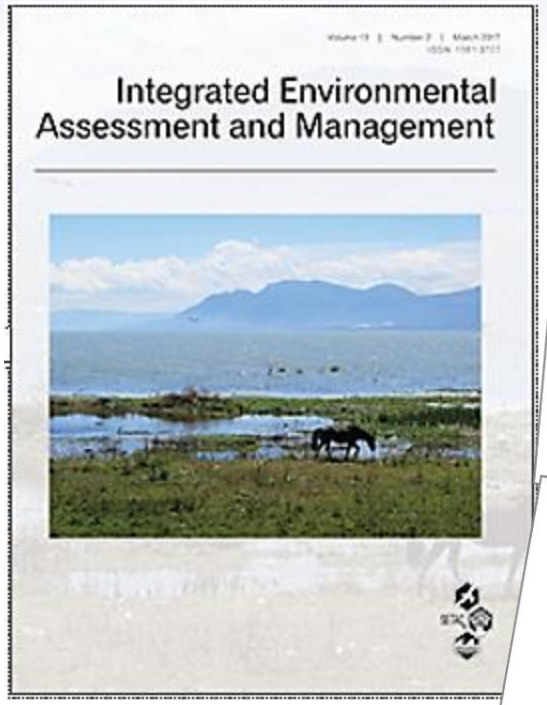
IF data do not allow proper scientific assessment (YET) a hazard based decision is scientifically justified

# Topics for Further Research

- Consideration of further endocrine pathways
- Test methods for under-represented taxa and pathways
- Methodological gaps for tiered progression
- Potentially sensitive (behavioural?) endpoints to link to population-level effects
- Determining adversity of effects
- Consider sensitive species/life stages/windows of exposure
- Methods to predict no-effect concentration/thresholds

http://onlinelibrary.wiley.com/doi/10.1002/ieam.v13.2/issuetoc

# Outcome




Special Series

Ecotoxicological Hazard and Risk Assessment of Endocrine Active Substances

Special Series

Challenges in Assigning Endocrine-Specific Modes of Action: Recommendations for Researchers and Regulators

Special Series

Uncertainties in Biological Responses that Influence Hazard and Risk Approaches to the Regulation of Endocrine Active Substances

Special Series

Current Limitations and Recommendations to Improve Testing for the Environmental Assessment of Endocrine Active Substa

Special Series

Population-Relevant Endpoints in the Evaluation of Endocrine-Active Substances (EAS) for Ecotoxicological Hazard and Risk Assessment

SETAC PELLSTON WORK

Environmental Hazard and Risk As

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IF data do not allow proper scientific assessment (YET) a hazard based decision is scientifically justified.

# Thank you for your attention!

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