*3°Conferenza AssICC – OCTIMA*
*Aggiornamento dei Regolamenti europei:*
*REACH, CLP, BPR (Biocidi)*

# *Gli interferenti endocrini (EDS): definizioni ed impatti sugli operatori*

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*ChemSafe Srl*

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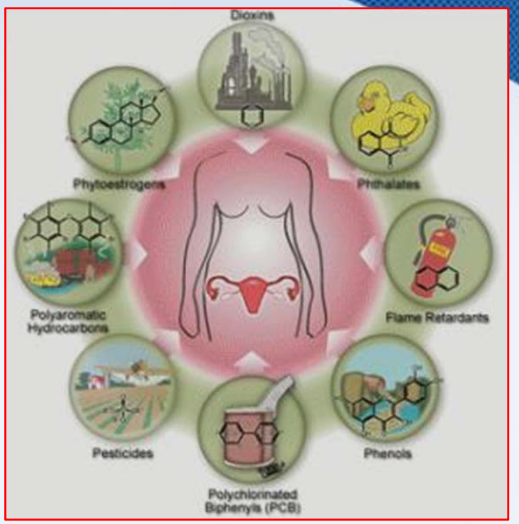
# SUMMARY




Introduzione e cenni storici

L'approccio scientifico

Le definizioni

Come si identificano, meccanismi di azione

Gli impatti sul diverse direttive/linee guida

(Fitofarmaci, biocidi, REACH, Farmaci)

Studi sperimentali (cenni)

Conclusioni

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## SCIENTIFIC DEFINITION

In general terms, the Endocrine Disrupting Chemicals (**EDC**) is a wide family of substances that may induce ***harmful effects*** to the organisms (human and/or animals) acting through an ***interference action*** within the ***hormonal (endocrine) system***. The Endocrine System is found in most animals, including mammals, non-mammalian vertebrates (such as birds, fish, amphibians, and reptiles), and invertebrates (such as snails and insects) and consists of a ***set of glands*** such as the thyroid, gonads, adrenal and pituitary glands, and the ***hormones they produce***, such as thyroxine, oestrogen, testosterone and adrenaline, which ***help***, ***guide*** and ***regulate*** the ***development***, ***growth***, ***reproduction*** and ***behaviour*** of animals, including human beings. Hormones are ***signal molecules*** which move through the bloodstream and elicit responses in other parts of the body

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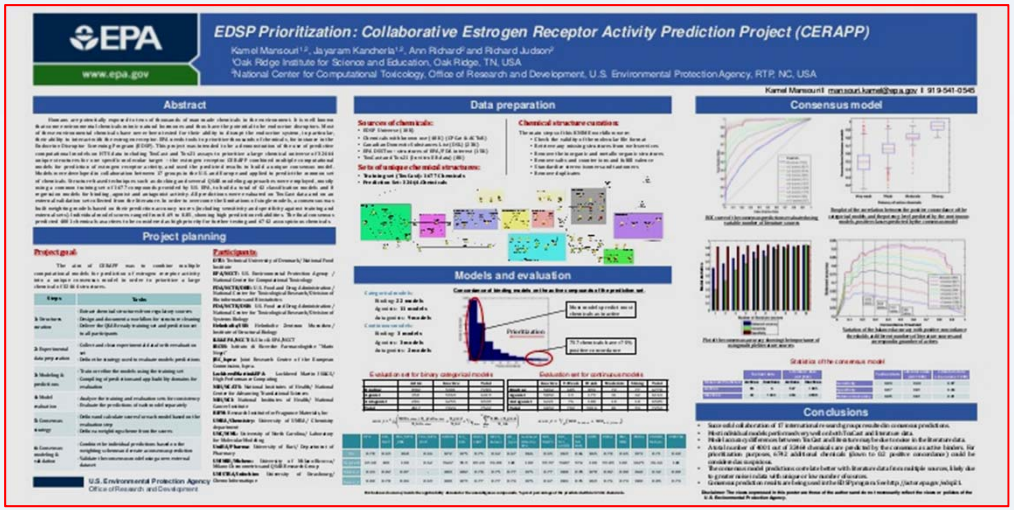
# CENNI STORICI

Iniziale interesse: anni ’90, molte pubblicazioni

*UK Royal Society of Chemistry*
*USA EPA (Environmental Protection Agency)*
*IPCS (International Program on Chemical Safety)*
*USA EPA EDSTAC (Endocrine Disruptor Screening and Testing Advisory Committee)*
*SETAC (Society of Environmental Toxicology and Chemistry)*
*OECD (Organization for the Economic and Cooperation Development)*
*ECETOC (European Center for Eco-toxicology and Toxicology of Chemicals)*

**USA EDSP** (Endocrine Disruptor Substance Programme)

1996 first list of potential EDS
(58 pesticide actives and 9 inerts)
nell’ambito del
**F**ood **Q**uality **P**rotection **A**ct


EPA
www.epa.gov
EDSP Prioritization: Collaborative Estrogen Receptor Activity Prediction Project (CERAPP)
Kamel Mansouri[1,2], Jayaram Kancherla[1,2], Ann Richard[2] and Richard Judson[2]
[1]Oak Ridge Institute for Science and Education, Oak Ridge, TN, USA
[2]National Center for Computational Toxicology, Office of Research and Development, U.S. Environmental Protection Agency, RTP, NC, USA
Abstract
Data preparation
Consensus model
Project planning
Models and evaluation
Conclusions
U.S. Environmental Protection Agency
Office of Research and Development

Grandi interrogativi:

***1.*** *cos’è un effetto avverso*
***2.*** *come possiamo identificarlo e valutarne i rischi*
***3.*** *come possiamo regolamentare gli EDS in una prospettiva sovranazionale*

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# DEFINIZIONI SCIENTIFICO/REGOLATORIE

**European Commission**: *"Endocrine disrupters are exogenous substances that alter function(s) of the endocrine system and consequently cause adverse health effects in an intact organism, or its progeny, or (sub)populations"*.

International Programme for Chemical Safety (IPCS): *"Endocrine disrupters have been defined as exogenous substances that alter function(s) of the endocrine system and consequently cause adverse health effects in an intact organism, or its progeny, or (sub)populations"*.

US EPA programme on endocrine disrupters: *"An exogenous agent that interferes with the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body which are responsible for the maintenance or homeostasis, reproduction, development and or behavior"* (Kavlock et al, 1996).

Japan (Ministry of Environment): *"Injury and/or hazardous effects on organisms caused by exogenous substances through influence on the endocrine system"*.

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## DEFINIZIONI SCIENTIFICO/REGOLATORIE per EFFETTO

An **adverse effects** is defined as *"a change in **morphology**, **physiology**, **growth**, **development**, **reproduction**, or, **life span** of an **organism**, **system**, or **(sub) population** that result in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress, or increase of susceptibility to other influences"* (WHO/IPCS 2009)

## COME SI IDENTIFICANO, Meccanismi di azione

**Mechanism of action**: a detailed molecular description of the mechanistic interaction through which a substance/molecule produces its effect. Normally considered at molecular level

**Mode of action (MoA)**: biologically plausible sequence of substance-specific key events, starting with exposure and proceeding with the interaction of the substance or its metabolites with a cell leading to an observed effect supported by robust experimental observations. A mode of action describes a functional or anatomical change at the cellular or biochemical level resulting from the exposure of a living organism to a substance

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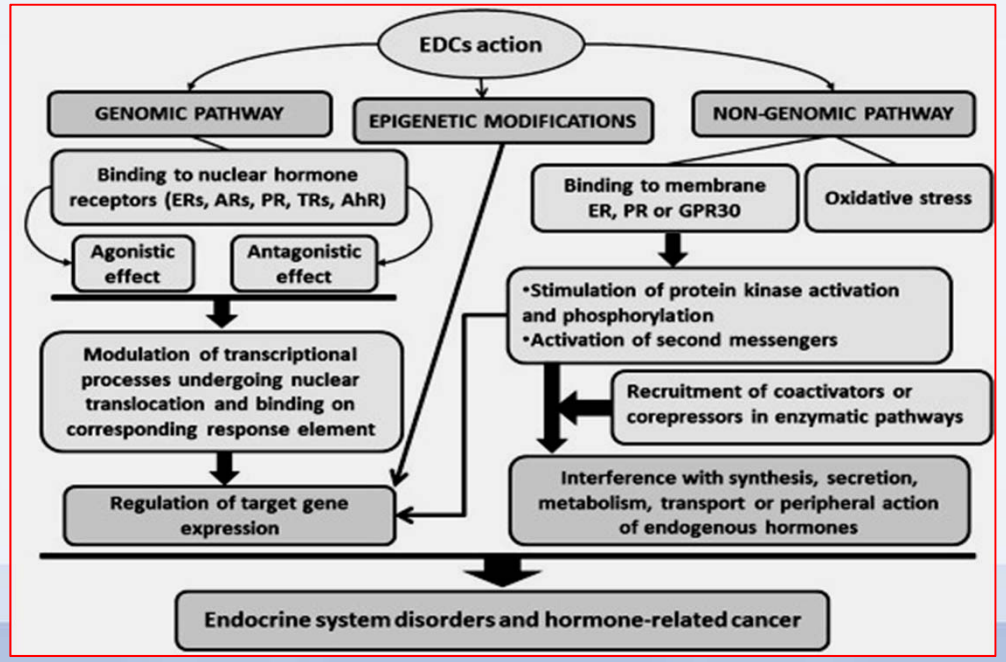
# TIPI DI INTERFERENZA

**Hormone mimicking** - mimicking a natural hormone, and thereby setting off similar chemical reactions in the body;

**Hormone receptor blocking** - blocking the hormone receptors in the cells, thereby preventing the action of the normal hormone;

**Hormone concentration alteration** – affecting the synthesis, transport, metabolism and excretion of natural hormones.

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## COME SI IDENTIFICANO

**Final guidance** of June 5, 2018 by joint EFSA and ECHA per biocidi e PPT

L'identificazione è focalizzata sullo studio delle **modalità EATS**
**EATS modalities** (*Estrogen, Androgen, Thyroid and Steroidogenic Activity*)

Devono essere soddisfatte tre condizioni base (tutte insieme): la sostanza risulta:

**a)** it shows an ***adverse effect*** in an intact organism or its progeny, which is a change in the morphology, physiology, growth, development, reproduction or life span of an organism, system or (sub)population that results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress or an increase in susceptibility to the influences;

**b)** it has an ***endocrine mode of action***, i.e. it alters the function(s) of the endocrine system;

**c)** the adverse effect is a **consequence** of the ***endocrine mode of action***.

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The crucial end-point of the evaluation is to establish a robust relationship/link between **adverse effects** caused by a substance and its **endocrine activity (Biological plausibility)**

ED assessment can be summarized in **five** steps :

**1.** Gather information

- *Data Search with Klimish rating for reliability*
- *Weight of Evidence (WoE)*
- *"in silico" prediction models*
- *"in vitro" studies*
- *"in vivo" studies*

**2.** Assess lines of evidence of adversity and endocrine activity
**3.** Initial assessment of the evidence
**4.** Mode of action analysis
**5.** Conclusion on the ED criteria

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## HUMANS and NON TARGET ORGANISM

The evaluation to determine if ED criteria are met need to be drawn separately for humans and non-target organisms; the hazard identification strategy must hence include two basic questions:

*Are there endocrine activity and adverse effect(s) relevant for **humans** which can be biologically plausible linked in an endocrine mode of action?*

*Are there endocrine activity and adverse effect(s) relevant for **non-target organisms** which can be biologically plausible linked in an endocrine mode of action?*

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# GLI IMPATTI SULLE DIVERSE DIRETTIVE/LINE E GUIDA

## Farmaci (veterinari ed umani)

Linea Guida ERA…. Per particolari classi di sostanze (ormoni, hormon-like) è necessario eseguire alcuni test per una prima verifica del potenziale EDS (FFLCT + hormonal end-points)

## Fitosanitari (PPT)

*Commission regulation **EU 2018/605** of April 19, 2018 amending Annex II to Regulation (EC) n. 1107/2009 by setting out scientific criteria for the determination of endocrine disrupting properties*

## Biocidi

*The COMMISSION DELEGATED REGULATION **EU 2017/2100** of September 4 2017 setting out scientific criteria for the determination of endocrine-disrupting properties pursuant to Regulation (EU) No 528/2012 of the European Parliament and Council was published on the European Official Journal on November 17, 2017 (n. L301)*

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## STUDI SPERIMENTALI

***Data/studies requested based on OECD Conceptual Framework (OECD CF)***

***Livello 1**: informazioni esistenti o informazioni non sperimentali*

| Mammalian and non mammalian toxicology | |
|---|---|
| *Level 1*<br>*Existing data and existing or new non-test information* | · *Physical & chemical properties, e.g., MW reactivity, volatility, biodegradability*<br>· *All available (eco)toxicological data from standardized or non-standardized tests.*<br>· *Read across, chemical categories, QSARs and other "in silico" predictions, and ADME model Predictions* |

## STUDI SPERIMENTALI

***Data/studies requested based on OECD Conceptual Framework (draft 2017)***

***Livello 2**: studi "in vitro" per lo studio del meccanismo d'azione (EATS modalities)*

| | |
|---|---|
| *Level 2*<br>*"In vitro" assays providing data about selected endocrine mechanism(s) / pathways(s) (Mammalian and non mammalian methods)* | · *Estrogen (OECD TG 493) or androgen receptor binding affinity (US EPA TG OPPTS 890.1150)*<br>· *Estrogen receptor transactivation (OECD TG 455), yeast estrogen screen (ISO 19040-1,2&3)*<br>· *Androgen receptor transactivation (OECD TG 458)*<br>· *Steroidogenesis in vitro (OECD TG 456)*<br>· *Aromatase Assay (US EPA TG OPPTS 890.1200)*<br>· *Thyroid disruption assays (e.g. thyroperoxidase inhibition, transthyretin binding)*<br>· *Retinoid receptor transactivation assays*<br>· *Other hormone receptors assays as appropriate*<br>· *High-Throughput Screens (See OECD GD No. 211 Describing Non-Guideline In Vitro Test Methods)* |

# STUDI SPERIMENTALI

***Data/studies requested based on OECD Conceptual Framework (draft 2017)***

***Livello 3**: studi "in vivo" per lo studio di specifici meccanismi di azione*

| | Mammalian toxicology | Non mammalian toxicology |
|---|---|---|
| *Level 3 "In vivo" assays providing data about selected endocrine mechanism(s) / pathway(s)* | · *Uterotrophic assay (OECD TG 440)*<br>· *Hershberger assay (OECD TG 441)* | · *Amphibian metamorphosis assay (AMA) (OECD TG 231)*<br>· *Fish short term reproduction assay (FSTRA) (OECD TG 229)2*<br>· *21 day fish assay (OECD TG 230)*<br>· *Androgenized female stickleback screen (AFSS) (GD 148)*<br>· *EASZY assay. Detection of Substances Acting Through Estrogen Receptors Using Transgenic cyp19a1b GFP Zebrafish Embryos. (draft OECD TG)*<br>· *Xenopus embryonic thyroid signalling assay (XETA) (draft OECD TG)*<br>· *Juvenile Medaka Anti-Androgen Screening Assay (JMASA) (draft OECD GD)*<br>· *Short-Term Juvenile Hormone Activity Screening Assay Using Daphnia magna (draft OECD TG)*<br>· *Rapid Androgen Disruption Adverse Outcome Reporter (RADAR) Assay (draft OECD TG)* |

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## *Data/studies requested based on OECD Conceptual Framework (draft 2017)*

### ***Livello 4**: studi "in vivo" per lo studio di effetti avversi rilevanti*

| | Mammalian toxicology | Non mammalian toxicology |
|---|---|---|
| ***Level 4***<br>*"In vivo" assays providing data on adverse effects on endocrine relevant endpoints* | · *Repeated dose 28-day study (OECD TG 407)*<br>· *Repeated dose 90-day study (OECD TG 408)*<br>· *Pubertal development and thyroid Function assay in peripubertal male rats (PP male Assay) (US EPA TG OPPTS 890.1500)*<br>· *Pubertal development and thyroid function assay in peripubertal female Rats (PP female assay) (US EPA TG OPPTS 890.1450)*<br>· *Prenatal developmental toxicity study (OECD TG 414)*<br>· *Combined chronic toxicity and carcinogenicity studies (OECD TG 451-3)*<br>· *Reproduction/developmental toxicity screening test (OECD TG 421). Combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (OECD TG 422)*<br>. *Developmental neurotoxicity study (OECD TG 426)*<br>· *Subchronic dermal toxicity: 90- day study (OECD TG 411)*<br>· *Subchronic inhalation toxicity: 90-day study (OECD TG 413)*<br>· *Repeated dose 90-day oral toxicity study in non-rodents (OECD TG 409)* | · *Fish sexual development test (FSDT) (OECD TG 234)*<br>· *Larval amphibian growth & development assay (LAGDA) (OECD TG 241)*<br>· *Avian reproduction assay (OECD TG 206)*<br>· *Fish early life stage (ELS) toxicity test (OECD TG 210)*<br>·*New guidance document on harpacticoid copepod development and reproduction test with amphiascus (OECD GD )201*<br>· *Potamopyrgus antipodarum reproduction test (OECD TG )242*<br>· *Lymnaea stagnalis reproduction )test (OECD TG 243)*<br>· *Chironomid toxicity test (OECD TG 218-219)*<br>· *Daphnia reproduction test (with male induction) (OECD TG 211)*<br>·*Earthworm reproduction test (OECD TG 222, 2004)*<br>*Enchytraeid reproduction test (OECD TG 220, 2004)*<br>· *Sediment water lumbriculus toxicity test using spiked sediment (OECD TG 225, 2007)*<br>· *Predatory mite reproduction test in soil (OECD TG 226, 2008)*<br>· *Collembolan reproduction test in soil (TG OECD 232, 2009)* |

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## STUDI SPERIMENTALI

***Data/studies requested based on OECD Conceptual Framework (draft 2017)***

***Livello 5**: studi "in vivo" per ulteriori dati per end-points rilevanti*

| | Mammalian toxicology | Non mammalian toxicology |
|---|---|---|
| *Level 5*<br>*"In vivo" assays providing more comprehensive data on adverse effects on endocrine relevant endpoints over more extensive parts of the life cycle of the organism* | · *Extended one-generation reproductive toxicity study (OECD TG 443)*<br><br>· *2-Generation reproduction toxicity study (OECD TG 416 most recent update)* | · *Fish lifecycle toxicity test (FLCTT)*<br>· *Medaka extended one-generation reproduction test (MEOGRT) (OECD TG 240)*<br>· *Avian 2 generation toxicity test in the Japanese quail (ATGT)*<br>· *Sediment water chironomid Life cycle toxicity test (OECD TG 233)*<br>· *Daphnia multigeneration test for assessment of EDCs (draft OECD TG)*<br>· *Zebrafish extended one generation reproduction test (ZEOGRT) (draft OECD TG)* |

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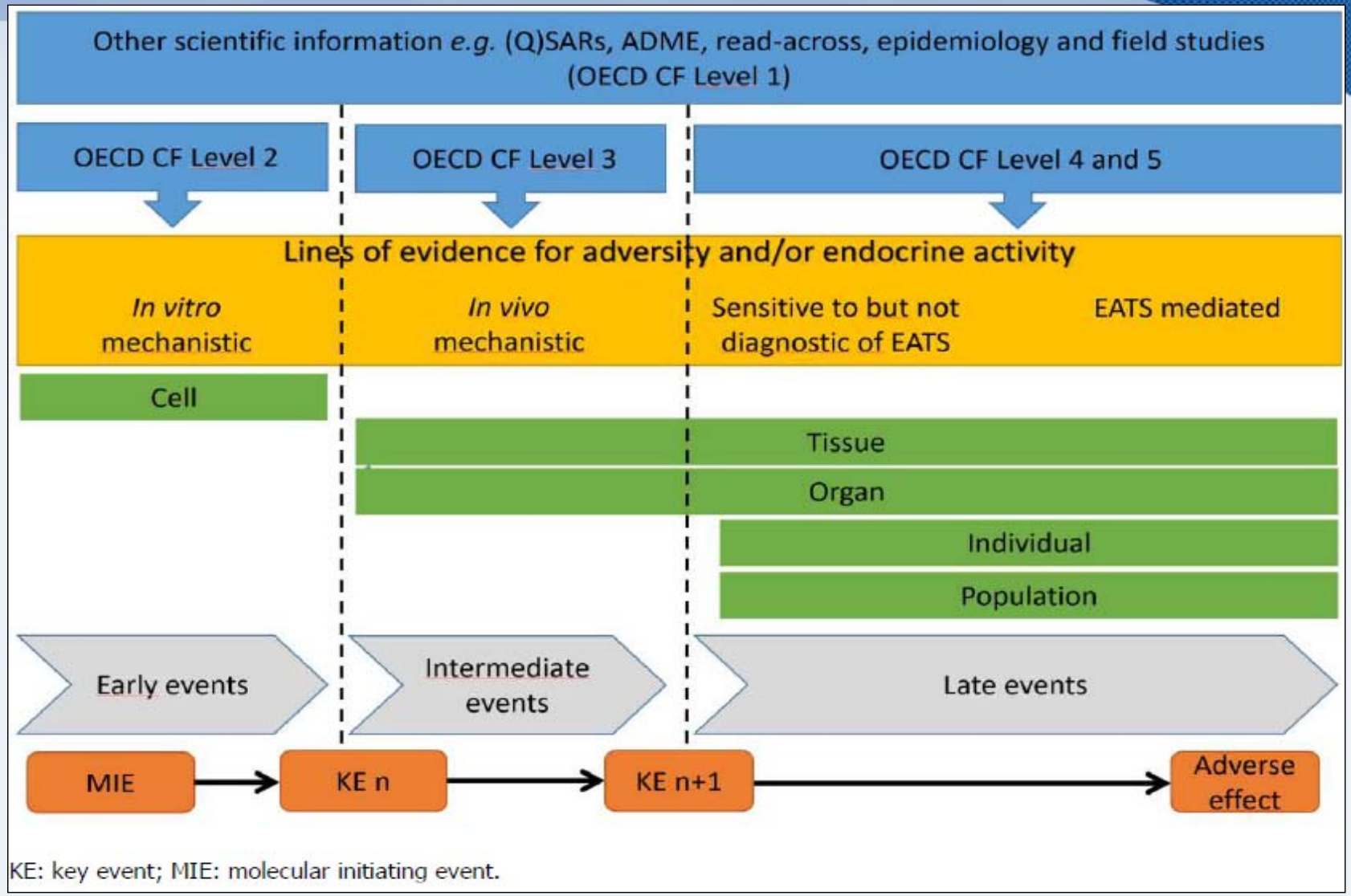


KE: key event; MIE: molecular initiating event.

# CONCLUSIONI (1)

**1.** Lo studio delle proprietà EDS è una attività complessa da punto di vista scientifico e regolatorio. Deve essere effettuata sulla sostanza attiva sia essa biocida, pesticida, farmaco o altro

**2.** Dopo 25 anni di discussioni non vi sono ancora comunque comuni vedute sulla problematica. Scarsa armonizzazione!!

**3.** L'approccio sperimentale è molto scientifico, step by step e pressuppone una conoscenza dei test molto approfondita

**4.** Lo studio del meccanismo di azione è difficile, necessità di un team multidisciplinare

**5.** Alcuni studi del pannello non sono ancora ufficialmente validati come OECD

**6.** Difficile stimare costi

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# CONCLUSIONI (2)

7. E' un problema di definizione del pericolo (Hazard Assessment) e non di definizione del rischio (Risk Assessment) pertanto non sono escluse procedure di autorizzazione per sostanze EDS

**Supply Chain**

**Business Continuity**

*Articolo 57*

**Sostanze da includere nell'allegato XIV**

Le sostanze seguenti possono essere incluse nell'allegato XIV secondo la procedura di cui all'articolo 58:

a) le sostanze che rispondono ai criteri di classificazione come sostanze cancerogene, categorie 1 o 2, a norma della direttiva 67/548/CEE;

b) le sostanze che rispondono ai criteri di classificazione come sostanze mutagene, categorie 1 o 2, a norma della direttiva 67/548/CEE;

c) le sostanze che rispondono ai criteri di classificazione come sostanze tossiche per la riproduzione, categorie 1 o 2, a norma della direttiva 67/548/CEE;

d) le sostanze che sono persistenti, bioaccumulabili e tossiche, secondo i criteri di cui all'allegato XIII del presente regolamento;

e) le sostanze che sono molto persistenti e molto bioaccumulabili, secondo i criteri di cui all'allegato XIII del presente regolamento;

f) le sostanze come quelle aventi proprietà che perturbano il sistema endocrino o quelle aventi proprietà persistenti, bioaccumulabili e tossiche o molto persistenti e molto bioaccumulabili, che non rispondono ai criteri di cui alle lettere d) o e), per le quali è scientificamente comprovata la probabilità di effetti gravi per la salute umana o per l'ambiente che danno adito ad un livello di preoccupazione equivalente a quella suscitata dalle altre sostanze di cui alle lettere da a) a e), e che sono identificate in base ad una valutazione caso per caso secondo la procedura di cui all'articolo 59.

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# CONCLUSIONI (3)

8. Non esiste un sistema di classificazione di sicurezza formale per EDS

9. Molte sostanze chimiche possono ricadere nella definizione di EDS quindi non bisogna sottostimare l'impatto di queste richieste.

10. Sostanze ritenute EDS possono causare ricadute di classificazione nelle miscele che le contengono.

11. **Mantenetevi aggiornati!!!**

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*Grazie per la vostra attenzione*

*Thanks for your attention*

*Gracias por su atención*

*Merci de votre attention*

*Danke fur ihre aufmerksamkeit*

*ChemSafe Srl*

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