# NEWSLETTER AUGUST 2018

## EDS (Endocrine Disrupting Substances), the final guidance

On June 4, 2018 the final guidance titled ***"Guidance for identification of endocrine disruptors in the context of Regulations EU n. 528/2012 and EC n. 1107/2009"*** was finally published and adopted by both ECHA (European Chemical Agency) and EFSA (European Food Safety Authority). The guidance is the outcome of a long discussion between stakeholders and a public consultation on the previous draft guidance ended on January 2018. The final guidance describes how to perform hazard identification for endocrine disrupting properties by following the scientific criteria which are outlined in Commission Delegated Regulation EU 2017/2100 *(see Chemsafe Newsletter November 2017)* and Commission regulation EU 2018/605 (April 19, 2018) for Biocidal Products )BP) and Plant Protection Product (PPP) respectively.
The guidance is addresses to BP and PPP only but in any case gives scientific principles and criteria on how to identify ED substances and it is considered a basic pillar of such evaluations in the future also in other fields.
The so called Endocrine Disrupting Chemicals, in brief EDC, is a wide family of substances, indeed not yet well defined, that may induce harmful effects to the organisms (human and/or animals) acting through an interference action within the hormonal system. Since mid '90s, the Scientific Community have been started the discussion on how to define and characterize the EDC activity and some Regulatory Bodies in USA as well as in Europe started to build up draft positive lists. From those years up to now such a discussion was kept alive by the debate between regulatory bodies and chemical/pharmaceutical industry; in 2006 the EDC substances have been mentioned in the REACH Regulation (EC1907/2006) within the group of the Substance of Very High Concern (SVHC). Recently the EDC category was also discussed within the regulations concerning Agrochemical and Biocides active substances and product in the frame of the cut-off criteria leading in some cases to additional special regulation publication. Regarding the pharmaceutical area, the assessment of a potential ED properties can heavily affect the ERA (Environmental Risk Assessment) of medicine when placed to the EU market firstly. The guidance is organized in **5 sections** and **7 Appendices** as follows.

Sections
***1.*** *Introduction*
***2.*** *Scope of the guidance document*
***3.*** *Strategy to assess whether a substance meets the endocrine disrupting criteria*
***4.*** *Information sources for Endocrine Disruptors Identification*
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Appendices
***A.*** *Additional consideration of how to assess the potential for thyroid disruption for human health*
***B.*** *Recommendations for design, conduction and technical evaluation of hormonal studies*
***C.*** *Information requirements for active substances under the BP and PPP Regulations which could potentially provide information on endocrine disrupting properties*
***D.*** *Data bases, Software tools and literature-derived (Q)SAR*
***E.*** *Excel template for reporting the available information relevant for ED assessment*
***F.*** *Examples on how to develop the search strategy protocol*
***G.*** *Example of MoA (Mechanism of Action) for non Target Organisms*

The evaluation requested by the guidance is essentially scientific as a sum of biology, endocrinology, toxicology, eco-toxicology and environmental fate approaches. In the following pages we will try to give the readers and overview of the approach without entering in so much scientific details.
Although the ED criteria cover all endocrine-disrupting mechanism of action (MoA) i.e. adverse effects which may be caused by any endocrine modality, the guidance document mainly addresses the effects caused by ***EATS modalities***. ***EATS stands for Estrogen, Androgen, Thyroid and Steroidogenic***. This is because the EATS modalities are currently the pathways for which there is a good mechanistic understanding of how substance-induced perturbations may lead to

adverse effects via endocrine-disrupting MoA. Additionally, only for EATS modalities there are at present standardized test guidelines for "in vivo" and "in vitro" testing available where there is a broad scientific agreement on the interpretation of the effects observed on the investigated parameters.
According to ED criteria of WHO/IPCS 2002, a substance shall be considered as having ED properties if it meets **ALL** the following criteria:

- *it shows an adverse effect in an intact organism or its progeny/non target organism, 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;*

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

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

Therefore, a substance has and endocrine disrupting mode of action when there is a **biologically plausible link between the adverse effect and the endocrine activity**. This assessment **must** be applied for humans and for non target organism.
When starting a ED evaluation a number of parameters (studies, data and information) may be available to be considered. It's necessary to order such parameters as for OECD GD 150 criteria to evaluate how they are relevant when investigating ED properties: They are grouped in four groups:
**1. "in vitro" mechanistic parameters**. They give information on the mechanism through which a substance could be considered endocrine active. Such parameters are currently placed under OECD CF (Concept Framework) **level 2**
**2. "in vivo" mechanistic parameters**. They provide information on endocrine activity that are usually not considered adverse. They are grouped under OECD CF **level 3**.
**3. EATS mediated parameters**. They are measured "in vivo" and contribute to the evaluation of adversity and considered indicative of EATS MoA. They are mainly under OECD CF **level 4 and 5**
**4. Sensitive to, but not diagnostic of, EATS parameters**. They are measured "in vivo" and may contribute to the evaluation of adversity, however, due to the nature of the effect and the existing knowledge, these effects cannot be considered diagnostic on their own of any of the EATS modalities (ex. altered stress responses).

**Assessment Strategy**
In brief, the assessment strategy can be summarized as follows:

**Step 1**
**Gather information**. Evaluate the relevance and reliability of all information obtained.
The applicant should provide all relevant scientific data which can give information on (potential) ED properties in the dossier. Data can include literature sources, studies "in vitro" and "in vivo", information from Read Across and (Q)SAR approaches as well as epidemiological data. All data must undergo a relevance and reliability (Klimish) assessment.

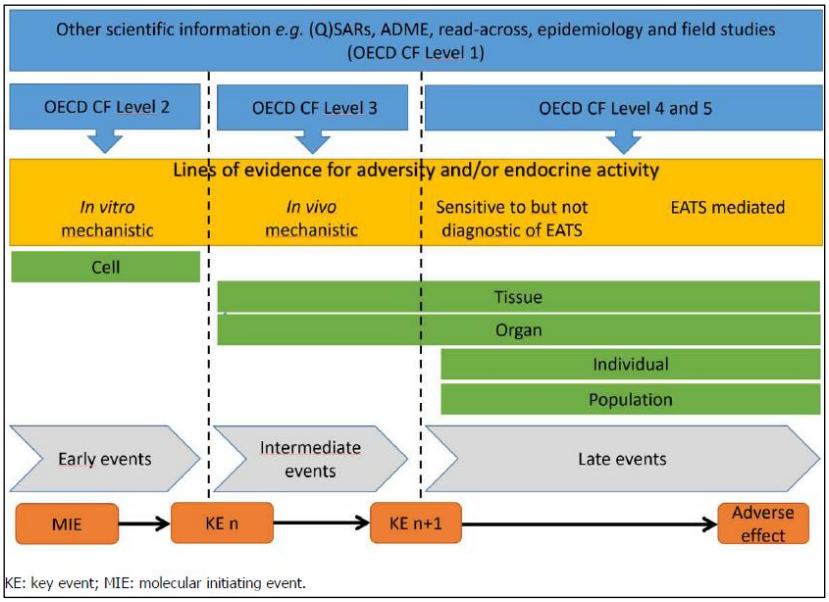
**Step 2**
**Assess the evidence**. The information are assembled in **lines of evidence** integrating information for both adversity and endocrine activity. A line of evidence is a broad term indicating *"a set of relevant information grouped to assess a hypothesis"*. Lines of evidence are not fixed and different subsets of information can be identified according to the contribution they made towards answering the problem formulated.

**Step 3**
**Initial analysis of the evidence**. This step include the assessment of different possible scenarios and it consists in making an analysis of the data set with respect to indication of EATS mediated adversity or EATS mediated endocrine activity. The analysis is made following an holistic approach and a Weigth Of Evidence (WoE) methodology on the available data/information. The outcome of step 3 can lead to consider the substance not meeting the ED criteria directly when no EATS mediated adversity is observed or, on the other side, to the need to generate additional information. In all other cases, it is necessary to postulate a **MoA** (Mechanism of Action), step 4, supported by a number of evidences/data/information and crucially by the plausibility for the link between the adverse effect(s) and the endocrine activity of the postulated MoA. If the information to support the postulated MoA are not sufficient additional information are needed or, alternatively, ED evaluation conclusion is not possible.

**Step 4**

**MoA (Mechanism of Action) analysis**. This step aims to establish if there is a biologically plausible link between the observed effects and endocrine activity by setting a MoA. A MoA can be described as a series of biological events, i.e. Key Events (KE) that results in the specific adverse effect. The MoA of an endocrine modality will normally contain some earlier KEs (which provides mechanistic information at the molecular or cellular level) and some later KEs (which provide mechanistic information at the organ or system level). To support and event as KEY, there needs to be sufficient body of experimental data in which the event is characterized and consistently measured. KEs are connected each other and this linkage is termed as Key Event Relationship (KER). The biological plausibility of each of the KERs in the MoA is the most influential consideration in assessing WoE in an overall MoA and it's weighted in three degrees: strong, moderate, weak. Other important factors to be evaluated are: essentiality, consistency, analogy and specificity.

**Figure n. 1** illustrates the approach to study the mode of action (adversity and endocrine activity) in relation of the OECD CF levels information/studies.




**Step 5**

**Conclusion on ED criteria**

The overall conclusion is based on the WoE elaborated to substantiate the postulated MoA. It is sufficient that the substance meets ED criteria for one group of non target organisms in order to be identified as ED. The conclusion of ED criteria needs to be transparently documented including the remaining uncertainties.

**Information sources for endocrine disrupting identification**

**Table n. 1**: Data/studies requested based on OECD Conceptual Framework, revised 2018

| Mammalian and non mammalian toxicology | |
|---|---|
| **Level 1** ***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 |
| **Level 2** ***"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 |

| | Mammalian toxicology | Non mammalian toxicology |
|---|---|---|
| **Level 3**<br>***"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) |
| **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<br>(OECD TG 222, 2004)<br>Enchytraeid reproduction test<br>(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) |
| | | |
| **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) |

Table n. 1 reports number of studies divided in three categories: available information and (Q)SAR data, "in vitro" studies, in vivo studies. Additionally studies are reported for mammalian and non mammalian in order to cover the assessment for humans and not target organisms. Most of tests are well established methods and already validated by OECD or equivalent bodies; some are still under validation and will add additional endocrine parameters. Some other tests have been validated by non OECD organizations. Non standardized test methods can also be used to derive relevant information provided that they are appropriately designed and judged to be of acceptable quality.

**Conclusion**

All of us surely share the ethical approach to protect the human health and the environment from ED chemicals so adversely and so sneaky in their action but industry is also concern about a possible disproportionate request from regulators which may lead to test a huge number of chemicals. Expenses to do so will create and unacceptable economic burden from industry; only large organization may bear it and, again, SME will be discriminated. Last but not least, both industry and regulators have to increase their assessment capacity hiring toxicologists that will need to have a broad knowledge including health regulatory toxicology, eco-toxicology, environmental fate processes, environmental biodegradation processes, secondary dietary risk assessment, specific adverse effects and so on in a frame of a more and more multidisciplinary and integrated approach. The reading of the presented guidance clearly demonstrates that the ED evaluation is a strong scientific activity including knowledge/understanding of biology, biochemistry, physiology, endocrinology, toxicology, eco-toxicology and environmental fate. Team of experts are hence needed to join together to judge this complex ED end-points.

A question remains: how many chemicals will be considered EDC? We do not know but we are only at the beginning of the story!!!

**Latest events**

Risk Assessment for Biocides
4-5 September 2018 - Chemical Watch - Brussels, Belgium

Regulatory Toxicology
10-11 September 2018 - Chem-Academy - Cologne, Germany/ NH Köln Altstadt

Classification of Mixtures
10-11 September 2018 - Chem-Academy - Cologne, Germany/ NH Köln Altstad

The Safety Data Sheet
17-18 September 2018 - Chem-Academy - Bonn, Germany/ Maritim Hotel Bonn

REACH Compliance for Downstream Users
18 September 2018 - Chemical Watch - Brussels, Belgium

Enforcement Summit Europe 2018
24-25 September 2018 - Chemical Watch - Belgium, Brussels
***Chemsafe attending with booth***

Preparing for Inspection Workshop
26 September 2018 - Chemical Watch - Belgium, Brussels

From Raw Material to Final Product Workshop
26 September 2018 - Chemical Watch - Belgium, Brussels

AsiaHub Summit Europe
27-28 September 2018 - Chemical Watch - Belgium, Brussels

*End of the Newsletter*