COMMENTS ON THE REGULATORY GUIDANCE DOCUMENT ON THE IDENTIFICATION OF ENDOCRINE DISRUPTORS FOR PESTICIDES AND BIOCIDES

BACKGROUND

Following a request by the European Commission, the European Agencies -European Food Safety Authority (EFSA), European Chemical Agency (ECHA)- with the assistance of Commission's Joint Research Centre (JRC), have produced a *draft* guidance document for identifying endocrine disruptors under EU legislation for pesticides and biocides.

The Guidance is providing an implementation strategy of the set of draft criteria that the Commission and Member States agreed upon[1], to identify pesticide and biocide substances with endocrine disrupting properties that may cause adverse effects in humans and non-target organisms (other than the target pest).

The Pesticide (EC 1107/2009) and Biocide (EC 528/2012) Regulations clearly state that **substances with endocrine disrupting properties for humans or non-target organisms should not be authorised**. Overall, the two Regulations are underpinned by the **precautionary principle** and emphasize that pesticide and biocide substances **shall have no harmful effects on human health, including of vulnerable groups, or animal health, or any unacceptable effects on the environment**.

PAN Europe has been an observer during the development of the document as EFSA stakeholder and provided comments internally during the expert group consultation. A final draft was published in December 2017. Below are the comments provided by PAN Europe provided in this last public consultation, which ended on 31st of January 2018.

https://comments.echa.europa.eu/comments_cms/PC_ED_Guidance.aspx

HIGHLIGHTS OF PAN EUROPE'S CONCERNS ON THE GUIDANCE

- In cases where there is evidence of adversity from animal experiments following exposure to a pesticide/biocide substance, the document seems to go into extreme detail to establish the plausible endocrine mode of action (MoA), through which the substance causes these adverse effects in humans and/or non-target organisms. This requires a high level of understanding of the function of the endocrine system across all species, which we currently don't have (due to lack of knowledge, lack of test methods, lack of data on existing test methods etc). Such approach may be useful for

[1] For the last version of the criteria go to https://ec.europa.eu/health/endocrine_disruptors/next_steps_en

the field of endocrine research but it is highly inadequate for regulatory risk assessment. In this type of assessment, a robust and straight-forward methodology is necessary to guarantee the protection of human, animals and environmental species from any harmful effects that pesticides and biocides may cause, no matter the mode of action.

- The guidance document appears to have a very narrow focus: the estrogen-androgen-thyroid-steroidogenic (EATS) modalities. Due to the lack of specific directions, in cases where non-EAST mediated adverse effects are observed, these may easily be dismissed from the evaluation as non-comprehensive. Here, we need to stress out that endocrine research has advanced and has identified endocrine hormones, production of hormones and hormone crosstalk in non-endocrine organs, such as adipose tissue, liver, muscle tissue, gut and the brain. Any observed adverse effects in these systems following exposure to pesticide or biocide chemicals are extremely relevant for regulatory purposes (obesity, diabetes, cognition deficiency etc), even if they don't have clear EATS modalities. The mandate[2] given to EFSA/ECHA is to produce a guidance document to identify all EDs not just the ones of EATS modalities.
- Even though developmental neurotoxicity and developmental immunotoxicity tests are included in the data requirements (but only as optional), it is unclear how these are evaluated since they don't necessary act through EATS modalities and no further guidance is given in this respect.
- The guidance document is incomplete as tests on invertebrate species are not compulsory and even when endocrine-related adverse effects are observed in these species, they do not trigger a conclusion that adverse effects have been observed on non-target organisms. Consequently, no regulatory action will suggested (e.g. negligible exposure, use in closed systems).
- Data gaps in GD are not correctly addressed producing a bias toward false negatives. In fact, due to the lack of data and scientific knowledge, it's far more likely to erroneously classify a substance as a non-ED, whereas it is extremely difficult to identify a substance as an ED even when the scientific evidence shows that it may cause ED-related adverse effects relevant for humans and/or non-target organisms. This is not in line with the regulatory requirements neither with the precautionary principle.

TECHNICAL COMMENTS

SECTION 2: SCOPE

Lines 188-189: Considering the current version of the document, here it should be clarified that it only provides guidance "*…on the implementation of the scientific criteria for the*

[2] https://ec.europa.eu/health/sites/health/files/endocrine_disruptors/docs/hazardbasedcriteria_mandate_en.pdf

determination of endocrine-disrupting properties only through EATS modalities limited to vertebrate species pursuant to Regulations…". However, given that the scope of the document is indeed much wider, PAN Europe emphasizes that the guidance should be modified in order to facilitate the incorporation of data on endocrine-related adverse effects from non-EATS modalities when there is available evidence and to also include adverse effects observed in non-vertebrate species, in line with the requirements of the European Regulations.

Lines 221-224: Please delete these last two sentences "*However, even though the revised version of the OECD GD 150 includes additional assays related to retinoid, juvenile hormones and ecdysterone modalities, no clear guidance on their interpretation is provided. Consequently, these additional assays currently do not allow any firm conclusions regarding endocrine MoAs.*" The pesticide and biocide Regulation mandate that substances should cause no adverse effects in humans and non-target organisms, no matter the mode of action.

Line 225: Delete "Nevertheless"

Lines 229-231: Start the sentence "The present document focuses on vertebrate (non-target) organisms…." and add the following sentence at the end: "Nevertheless, data on adverse effects on invertebrates from invertebrate test assays or field data, assumed to be endocrine-related, may provide information that can be used in decision-making, despite the lack of knowledge on the MoA".

Lines 229-231: Delete the whole paragraph.

SECTION 3: STRATEGY

Line 261: According to the WHO/IPCS definition (WHO/IPCS 2002) an endocrine disruptor is a substance "…that alters the function(s) of the endocrine system and consequently causes health adverse effects...". Although we understand that the definition is divided to three sections for practical reasons (endocrine activity, adverse effect, and plausible MoA between the two), this division should not result in an oversimplification of the endocrine system. An alteration of the function of the endocrine system is not a single endocrine action and should not be perceived as such. Its an orchestrated action that involves several organs, hormone crosstalks and receptor coactivations. Further, the most important element in hazard assessment is the development of adverse effects following exposure to the substance under investigation, and this should be emphasized in this section.

Lines 277-279: Delete the word "firm" from "firm conclusion". Conclusions on pesticide toxicity cannot be considered absolute (firm) and that is why they are revised every 10-15 years. In line 278, after ED criteria add "based on all available data"

SECTION 3.1 STRATEGY OVERVIEW

Lines 324-326: One obvious problem with this division apart from focusing solely on EATS modality (and further with the assessment strategy) is that other non-EATS adverse effects are excluded. Even the developmental neurotoxicity and immunotoxicity adverse effects (cohorts 2 and 3), which are supposedly requested in data requirements, are not properly addressed here as these effects don't necessarily have EATS modalities. An indication on what action will be triggered if NDT and IDT endpoints show adverse effects is missing and should be included.

Line 334 (in vitro): At the end of the paragraph include "The results of L2 assays are considered preliminary as they lack metabolic and feedback systems".

Line 347 (in vivo mechanistic): At the end of the paragraph include "These assays are of short duration and do not reveal the full spectrum of potential adverse effects"

Line 350 (sensitive to, but not diagnostic of, EATS): Here it should be emphasized that since these effects are adverse, they are still relevant for the hazard assessment of pesticides/biocides. Include after potentially adverse "and therefore are still relevant for the overall hazard assessment of pesticides and biocides".

Lines 371-374 (assessment strategy): Here a clarification is missing that if adversity is observed in parameters considered "sensitive to, but not diagnostic of, EATS" are still of concern and therefore relevant for the pesticide risk assessment.

Lines 402-403: The figure is missing to indicate what happens when endocrine related adversity due to non-EATS modalities is detected. Also, since systematic review includes evaluating data quality and assembling the lines of evidence it seems it should be placed further down.

Line 406: These "other data" are not defined in the glossary of terms. Please consider defining as it is unclear to what data you refer.

SECTION 3.2 GATHERING ALL RELEVANT INFORMATION

Lines 13-14: Specify that all mandatory studies should be carried out according to the latest version of the corresponding guideline (as it's already stated in section 3.2.2.1 lines 96-99).

Lines 91: Write "updated EU test methods" as old protocols may not be relevant, even if they have included endocrine disruption endpoint (e.g. they may have been carried out in adults).

SECTION 3.3 ASSEMBLING LINES OF EVIDENCE

Lines 264-267: In certain cases this analysis is redundant and creates a risk of misinterpretation of observed adverse effects.

Lines 321-322: Here add an extra sentence at the end for clarification: "It should be noted that adverse effects may be specific to the time and duration of exposure, and may be different in juveniles or foetuses than adults. Therefore, comparison of studies for consistency should be done following expert's judgement"

SECTION 3.4 INITIAL ANALYSIS OF EVIDENCE

Lines 376-378: Here it seems scientifically unfounded to claim that EATS mediated adversity parameters reported in all other scientific studies other than OECD TG 443 (including non-TG studies from academic scientific literature) will be considered not to be sufficiently investigated. Consider rephrasing.

Line 377: after TG 443, write "with cohorts 2 and 3 for NDT and IDT"

Lines 378-379: In principle if 'EATS-mediated' adversity has not been investigated this should be considered as data gap - updated level 4 & 5 tests are in the data requirements of pesticide and biocide regulations.

Table 4. This table is oversimplified as it leaves room for misinterpretation of data and should therefore be modified. A detailed analysis is given in the text.

Line 405 (3.4.1): If TG 443 (with 2 and 3 cohorts) comes out negative but there is EATS mediated adversity indicated in other TGs or there are non-EATS mediated endocrine-related adverse effects have been observed, these would also be relevant for regulatory purposes. Scenarios 1 need modification.

Lines 416-117 (scenario 1a): Here, if the body evidence does not show any EATS mediated adversity (L4 and 5 updated TG and independent literature) this scenario should say "it does not meet the ED criteria through EATS modality with regard to humans" but other adverse effects from non-protocol studies may be relevant for hazard assessment.

Lines 424-427 (scenario 1b): In line 425, after a "MoA analysis" delete "is required…" and add "in principle is not necessary as the evidence may already be sufficient to conclude the plausible biological link between the adverse effect and endocrine activity". Adverse effects in apical endpoints observed in TG 443 (with the two cohorts) should be evaluated as hazard by default. The same is true for non-target organisms and TG 240 & 241.

Lines 434-436 (scenarios 2a): Here, the meaning of "NO" (not sufficiently investigated) is extremely vague. A list of the minimum set of endocrine-relevant data (e.g. AGD, nipple retention, VO, PPS, oestrous cyclicity) or relevant endpoint from non-standardised protocols should be given, otherwise it becomes highly hypothetical to make any decision. At least from protocol studies, the GD should provide the minimum of parameters necessary to accept Level 4/5 studies as adequate for the assessment or otherwise the data requirements would be incomplete and L5 studies should be repeated/performed (TG 416, TG 443). Make

a specific reference to tests for neurotoxicity and immunotoxicity as they are not necessarily EATS mediated, but are included in the data requirements.

Line 454-56 (scenario 2ai): Please delete and add instead: **"**As not all 'EATS-mediated' parameters have been investigated, additional missing information on adversity in vivo needs to be generated to enable MoA analysis."

Line 460 (scenario 2aii): Here it is crucial to know what EATS parameters have been investigated in vivo, from standard protocols and independent literature as apical effects may indicate endocrine related endpoints that are not captured by L2 and L3 tests. L3 and L2 tests may not be sufficient without data from in vivo studies

Line 461: Rephrase as follows "If the available/generated mechanistic information from all scientific literature does not give indication of endocrine disruption then".

Line 466: After conducted add "and metabolic activation must have been properly assessed;"

Line 467: Add at the end of the line, after that "it is likely that"

Line 468: rephrase as "does not meet the ED criteria through EATS modality for humans and non-target organisms". Add at the end "Nevertheless, a decision is not possible on non-EATS endocrine related adverse effects, neurotoxicity and immunotoxicity".

Lines 475 (scenario 2aiii): Here again it is crucial to know what EATS parameters have been investigated in vivo, from standard protocols and independent literature as apical effects may indicate endocrine related endpoints that are not captured by L2 and L3 tests.

Line 477: Remove "or", both L2 and L3 tests should be performed including metabolic activation.

Line 490 (scenario 2b): Here depending on the EATS mediated parameters identified on adversity, the plausible link could be directly reported, together with consistency of the effects and no MoA analysis is necessary.

SECTION 3.5 MODE OF ACTION ANALYSIS

Lines 499-504. For PAN Europe the MoA is not necessary to investigate further when L4 and L5 tests, or similar non-protocol studies, conclude endocrine-related adverse effects. Particularly L5 tests are designed (or updated in the case of TG 416) to detect adverse effects through alterations in the endocrine system and it seems redundant to continue carrying out the analysis. The information on any endocrine-related adversity is sufficient to withdraw or refuse the authorisation of a pesticide or biocide substance according to the European Law (which is also based on the precautionary principle).

Lines 597-599: Please rephrase and specify that "If no adversity is observed in L4 and L5 updated guidelines, and other relevant non-guideline studies, this would support the lack of an EATS endocrine mode of action in vivo". This is also the proposal of the TG 150. If there is endocrine activity, it is not sufficient to investigate only L3 tests. On the other hand, if endocrine activity is detected and adversity is observed in L3 tests or similar non-protocol tests, then it is likely that the substance is an ED.

Lines 623-626: This is a key sentence in line with the requirements of the regulation and should apply at least in scenarios 1b, and 2b. "*In these scientific frameworks the level of evidence required to support the sequence of events leading to adversity might be considered too high a requirement for the hazard identification of an ED for regulatory purposes (JRC 2013). To conclude on the biological plausibility of the link, it may not be necessary to establish the whole sequence and relationship of events leading to the adverse effect.***"**

Line 642-643: Here the biological plausibility of the link between two KEs is redundant, considering that a biological link has already been established through adverse effect and endocrine activity (and of course exposure). Delete "and secondly the biologically plausible link between two KEs".

Line 674: Delete "between, for example, the KE up and the KE down"

Line 682: Rephrase as follows: "The **amount of evidence** of the biological plausibility is weighted as follows". If KEs are not understood that doesn't mean that the biological plausibility is weak but that the body/amount of evidence on biological plausibility is weak.

Lines 688-727: This section is based on assumptions and should be removed. The international protocols, including the conceptual framework on EDs produced by OECD are not designed to address the parameters of dose response across all studies and temporal concordance. Any endocrine-related adverse effect should be relevant. Exposure to endocrine disruptors during different points of life time may give rise to different apical effects, through a different pathway of KEs. Our knowledge on these endocrine mechanisms at different life times in vivo is very limited and the data we have are not usually comparable (long term, short term, in vivo, in vitro). Data should be treated as body of evidence even if the responses do not follow a dose response or temporal concordance.

Lines 728-772: This section could easily lead to equivocal assumptions due to the lack of adequate data that assess endocrine disruption. The essentiality, consistency, specificity and analogy of KEs goes beyond the requirements of regulatory risk assessment. Endocrine-related adverse effects in animal experiments following exposure to a pesticide/biocide substance should be considered unacceptable, whether our current scientific knowledge allows the identification of the KEs and their relations or not.

Lines 863-864 (section 8.6.3): This sentence does not make sense, if a substance has an endocrine activity and causes apical adverse effects in systems that investigate endocrine disruption (animal experiments), then the most likely explanation is that our current knowledge of endocrinology is not sufficient to understand the link, rather than assuming that there is no link at all. Delete the sentence.

Lines 911-912: This is a very important point and should be developed further throughout the document. Clear guidelines on how to include non-EATS modalities should be given. When such data exist, they should be incorporated in the risk assessment to avoid classifying a hazardous substance as safe.

SECTION 4

This section should include at least some tests on non-vertebrates (invertebrate species) as they are relevant for the European regulation, according to which substances that have endocrine disrupting properties that may cause adverse effects in humans and non-target organisms should not be authorised.

SECTION 5 RECOMMENDATIONS

Line 2202 (5.1): These seem basic scientific principles in the performance of assays, it is not clear why they are included as recommendations and not as guidelines. What happens when these recommendations are not fulfilled? Will the assays still be valid?

Contact:

Angeliki Lyssimachou, PhD. Environmental toxicologist

Email : angeliki@pan-europe.info