ENDOCRINE SOCIETY
Hormone Science to Health

European Food Safety Authority
Via Carlo Magno 1A
43126 Parma
ITALY

April 28, 2016

Regarding: Public consultation on Guidance for the identification of biological relevance of adverse/ positive health effects from experimental animal and human studies

Dear Members of the EFSA Scientific Committee,

The Endocrine Society appreciates the opportunity to comment on the "Public consultation on Guidance for the identification of biological relevance of adverse/ positive health effects from experimental animal and human studies." Founded in 1916, the Endocrine Society is the world's oldest, largest, and most active organization dedicated to the understanding of hormone systems and the clinical care of patients with endocrine diseases and disorders. Our membership of over 18,000 includes researchers who are making significant contributions to the advancement of knowledge in toxicology, especially in the field of endocrine-disrupting chemicals (EDCs).

The Endocrine Society has consistently argued that the current regulatory apparatus in the European Union is inadequate for the evaluation of health effects due to EDC exposures. Upon a careful review of the draft guidance document, we make several recommendations to improve the guidance so that risk assessors are better equipped to evaluate the biological relevance of effects due to exposures to EDCs. Our detailed suggestions are listed below, and have been submitted separately via the on-line form.

Thank you for considering our comments. If you have any questions, or if an expert member of the Endocrine Society can be of further assistance in your efforts, please feel free to contact Joseph Laakso, PhD, Associate Director of Science Policy at jlaakso@endocrine.org.

Sincerely,

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Summary - Lines 34-37: As written, the third bullet implies that there must be a monotonic dose-response that reaches a threshold to become adverse. We urge EFSA to explicitly include consideration of non-monotonic dose responses, as these types of responses are observed with many effects of EDCs[1].

Summary - Line 40: Interpretation of the fourth bullet point is complicated by a truncated sentence; however, we assume that this sentence states that the conditions of exposure should be considered. We note that conditions of exposure can change faster than assessments are updated, and not all conditions of exposure can be immediately foreseen. Additionally, highly hazardous chemicals, or hazardous chemicals where there is also the potential for exposure to vulnerable populations such as pregnant women and infants, should be given special consideration irrespective of the level of exposure. We therefore recommend that the guidance document reflect that assessments should evaluate both the hazard and risk of a chemical.

Introduction - Lines 120-123: We note that distinguishing adverse effects from physiologically adaptive effects is very difficult to do through toxicological assays, especially when such assays are not reflective of real world situations.

Section 3.1.2. – General: The Endocrine Society is concerned that multiple, partially overlapping evidence streams for Adverse Outcome Pathways (AOPs), Mode of Action (MOA) and mechanistic information are used in the draft guidance, and that it may frequently be the case that comprehensive information is lacking in one or more of these evidence streams. We assert that these evidence streams should be used in such a way that they will increase the confidence and efficiency of regulatory decision-making for those chemicals with high hazard or well-documented adverse effects, and that gaps in our understanding of an AOP, MOA, or mechanism should not prevent or slow progress for regulatory processes for such chemicals.

Section 3.1.2 - Lines 439-445: As written, the concept of biological relevance relies on AOPs as one way to determine if the underlying molecular pathway is relevant. However, it should be noted that many of the steps in AOP pathways for new chemicals, or even those chemicals with established adverse effects, are not characterized to the extent that would be definitively reliable to determine biological relevance. Many pathways needed to demonstrate biological relevance for EDCs cannot be described by a simple linear approach, as they would need to reflect complex, branched interactions involving multiple biological systems. These complex signaling webs could not be sufficiently addressed under the current methodology on AOP construction. Furthermore, the magnitude of a molecular effect should not be considered as a strict requirement of biological relevance. As reflected in line 438, a small initial molecular effect can set off a chain of molecular events that can result in a significant adverse effect, as has been shown with pharmaceutical agents.

Section 3.1.2 - Lines 446-468: As defined in the draft guidance, MOA, mechanism of action, and AOPs contain overlapping elements. For instance, if the definition of MOA states that it requires robust experimental

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[1] Zoeller, RT, et al., "Endocrine-Disrupting Chemicals and Public Health Protection: A Statement of Principles from The Endocrine Society." *Endocrinology* (2012) 153 (9): 4097-4110.

observation and mechanistic data to describe a biologically plausible sequence of key events leading to an observed effect, how do the uses of the systems differ in regulatory decision making and are the evidence streams duplicative? Case studies would be particularly helpful, showing how this evidence is incorporated.

Section 3.1.2 - Lines 469-471: The definition of AOP supplied in the guidance requires that it be of regulatory concern and states that the events should be definable and make physiological and biological sense within a toxicity pathway. We assert that this should not preclude evaluation of the many indirect actions of chemicals, where complex webs of indirect actions may have an impact on toxicity, resulting in an adverse outcome. It is unclear how the simplistic definition provided in the guidance will be suitable for real world assessment of the risk of a chemical.

Section 3.1.3 - Lines 510-516: The definition of thresholds implies that toxicological dose response curves may only be monotonic; however, there is demonstrable evidence that non-monotonic curves are common e.g., for EDC effects[2]. This sentence should be reworded to consider the possibility of such effects.

Section 3.1.5 - Lines 623-624: In the discussion on models, it is important to clarify the phrase "as long as they are based on sound approaches and explicit assumptions." Models used to inform risk assessments should be transparent and open for the public and scientific community to evaluate, including information on assumptions used and statistical/mathematical treatments of the model.

Section 3.1.6 - Lines 645 - 649: This section states that a biomarker of effect does not indicate whether an effect is adverse or not, and this depends on its biological relevance which is indicated through mode of action and adverse effect or AOP. Though relation to MOA and/or AOP can certainly indicate that an effect is adverse, a biomarker that does not signal an AOP sufficiently could still indicate adversity through another mechanism not reflected in an AOP pathway. In complicated systems, simply relying on an AOP to determine if an effect is adverse could be misleading. In situations where little is known about off-target pathways, indirect effects, or where science indicates that an adverse outcome could occur through other pathways, a lack of relation to MOA or AOPs should not preclude the utility of the biomarker in question.

Section 3.2.1 - Line 695: In addition to the preceding questions, we recommend that an additional question be added – what information is missing or would be useful to make a more informed assessment of this chemical? The approach laid out in lines 680-694 assumes that only guideline information will be used to assess the chemical. However, the use of additional information in peer-reviewed scientific literature may provide highly relevant scientific information beyond what the guideline studies can provide. Regulatory bodies and assessors should be encouraged to stay informed of and use peer-reviewed literature, which frequently can fill gaps in knowledge missing from guideline studies or identify improvements to those studies.

Section 3.2.3 - Line 792: This statement should include a reference to non-monotonic dose responses.

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[2] Gore AC et al., "EDC-2: The Endocrine Society's Second Scientific Statement on Endocrine-Disrupting Chemicals." *Endocr Rev*. 2015 Dec;36(6): E1-E150.

Section 3.2.3 - Lines 854-859: This section should include a statement on the relevance of non-monotonic dose responses.

Section 3.2.3 - Lines 876-880: The example of eggshell thinning highlights two issues that are not adequately addressed by the guidance:

- If hatching and survival is considered the biologically relevant endpoint, then thinning up to 18% may not be considered adverse for a single chemical. However, thinning of these eggs could be considered adverse in the context of other endpoints, and/or they may be more susceptible to the effects of other stressors including other chemicals which may have different endpoints not captured by hatching or survival.
- Complicated biological endpoints are routinely impacted by multiple chemical exposures and at different developmental windows. Additive effects of chemical exposures should be considered, and this should be reflected in the guidance.

Therefore, when such effects are observed, they should be considered potentially adverse because the definition of adversity is limited by a one-chemical one-pathway approach. As described in the guidance, modelling only emphasizes the one chemical approach, which is often not sufficient to explain real-world exposure scenarios. We recommend that modelling of cumulative and/or mixture effects be discussed in this section.

Section 3.2.3 - Line 978: Figure 4 should include a pathway allowing for the evaluation of indirect effects, for example by asking the yes/no question that phenylalanine would lead to an adverse effect if accompanied by additional stressors.