Glyphosate has been concluded to not meet the criteria of endocrine disruptor in the draft RAR

The ECHA/EFSA Guidance from 2018, for the identification of endocrine disruptors, identifies an endocrine disruptor as *"[…] as an active substance shall be considered as having endocrine disrupting properties that may cause adverse effects to (non)-target organisms if it is a substance that (1) shows an adverse effect in (non)-target organisms, (2) had an endocrine mode of action (MoA), and (2) that adverse effect is a consequence of the endocrine MoA*."

Therefore, there must be a plausible (i.e., causal) link between an endocrine MoA and an adverse effect to conclude that an active substance has endocrine disrupting properties (Figure 1). Without this linkage, between an endocrine MoA and an adverse effect based on a weight of evidence assessment, an active substance cannot be concluded to be an endocrine disruptor.

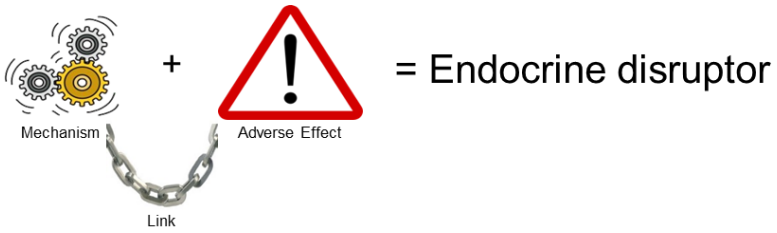

Figure 1. There must be causal link between an endocrine MoA and an adverse effect based on a weight of evidence to conclude that an active substance is an endocrine disruptor.

Identification of a potential endocrine disruptor under the ECHA/EFSA Guidance requires an assessment of potential interaction with the estrogenic, androgenic, thyroid, and steroidogenic (i.e., EATS) pathways. Therefore, an endocrine assessment often includes mechanistic data from *in vitro* assays and mechanistic and apical data from *in vivo* studies that have been designed to include endpoints that inform an of the EATS pathways. In addition, a weight of evidence evaluation includes data from standard toxicology and ecotoxicology studies that include endpoints that can inform an endocrine assessment as well as the relevant and reliable information from the literature that can further inform an endocrine assessment. Glyphosate is considered to be a data-rich active substance in terms of the availability of assays that evaluate EATS pathways.

Glyphosate was first evaluated for its potential to be an endocrine disruptor under the United States Environmental Protection Agency's (USEPA) Endocrine Disruptor Screening Program (EDSP) and then by the European Food Protection Agency (EFSA) as part of the Annex 1 renewal for glyphosate (USEPA, 2015; EFSA, 2017). In addition, glyphosate was recently evaluated for its potential to interact with the endocrine system and published in the peer reviewed literature (Levine et al., 2020). Most recently, as part of the ongoing glyphosate re-evaluation in the EU, glyphosate was evaluated ne more by the RMS and included in the draft Renewal Assessment Report.

The recent endocrine assessment for glyphosate can be found in dRAR under **section 2.10** (page 708) titled "Endocrine Disrupting Properties" with the overall conclusion under section **2.10.4** for both the human and non-target organism assessments. For the complete volume covering the human assessment please refer to the separate document "Volume 1, 2.10.2 ED assessment for Humans".

To meet the requirements the USEPA's EDSP, eleven Tier 1 assays were submitted in 2012 to evaluate potential disruption of the estrogen, androgen and thyroid pathways including steroidogenesis. In addition, potential effects on the hypothalamus-pituitary-gonadal and hypothalamus-pituitary-gonadal axes were evaluated to detect potential impacts on apical endpoints such as pubertal development and reproduction. The results from the Tier 1 EDSP battery should no interaction with EATS pathways (Table 1). The EDSP results are supported by the large toxicology and ecotoxicology database for glyphosate as well as by the relevant and reliable information from the literature that provides data of the same nature and quality as EDSP battery below.

Table 1. Results from the EDSP Tier 1 assays, and the modes–of–action they evaluate, showing no interaction with EATS pathways. A "NO" in each column below signifies the assay was negative for the different MoAs.

	Modes–of–Action (MoA)							
	Receptor Binding				Steroidogenesis			
Screening Assay	E[1]	Anti–E	A[2]	Anti–A	E	A	HPG[3] Axis	HPT[4] Axis
ER[5] Binding	No	No						
ERTA[6]	No							
AR[7] Binding			No	No				
Steroidogenesis					No	No		
Aromatase					No			
Uterotrophic	No							
Hershberger			No	No		No		
Pubertal Male			No	No		No	No	No
Pubertal Female	No	No			No		No	No
Amphibian metamorphosis								No
Fish short term reproduction	No	No	No	No	No	No	No	

[1] Estrogen; [2] Androgen; [3] Hypothalamic Pituitary Gonadal; [4] Hypothalamic Pituitary Thyroid; [5]Estrogen Receptor; [6] Estrogen Receptor Transcriptional Activation; [7]AR = Androgen Receptor.

The USEPA's 2015 and the EFSA 2017 weight-of- evidence evaluations considered the EDSP studies, glyphosate toxicology studies that informed an endocrine assessment (e.g., rat multi-generational reproduction studies), and relevant and reliable studies from the open literature that further informed an endocrine assessment. The strength these weight of evidence evaluations was that they included data across multiple levels of biological organization (i.e., subcellular, cellular, organ, and whole organisms), tested mammalian and non-mammalian species, and the majority of the studies followed validated regulatory test guidelines (e.g., EPA OCSPP and/or OECD test guidelines).

Based on the weight-of-evidence from these evaluations, that considered the EDSP assays, a comprehensive toxicology database and the relevant literature studies, it was concluded that exposure to glyphosate does not have the potential to interact with the endocrine system and will not result in adverse effects through an endocrine mechanism (i.e., glyphosate does not have endocrine disrupting properties). Recently, as part of the EU glyphosate renewal, glyphosate was re-evaluated under the new EFSA/ECHA

guidance for assessing endocrine disruption. The conclusion in the draft RAR is consistent with the previous EPA and EFSA evaluations - - glyphosate does not induce EATS-mediated adversity and no EATS-related endocrine activity was observed *in silico*, *in vitro,* and *in vivo*.

The USEPA's and EFSA's conclusions for 2015, 2017 and 2021 are provided below.

EPA's conclusion (2015)
"*Based on weight of evidence, EDSP Tier 2 testing is not recommended for glyphosate since there was no convincing evidence of potential interaction with the estrogen, androgen or thyroid pathways*."

EFSA's conclusion (2017)
"*The weight of evidence indicates that glyphosate does not have endocrine disrupting properties through oestrogen, androgen, thyroid or steroidogenesis mode of action based on a comprehensive database available in the toxicology area. The available ecotox studies did not contradict this conclusion*."

dRAR conclusions (2021)

Humans
"In conclusion, glyphosate does not induce EATS-mediated adversity and no EATS-related endocrine activity was observed in silico, in vitro, and in vivo. This conclusion is in concordance with the current peer review of the pesticide risk assessment of the potential endocrine disrupting properties of glyphosate (EFSA Journal 2017;15(9):4979) as well as with the conclusion of EPA on the Endocrine Screening Program (EDSP) Tier l (US EPA, 2015)."

Mammalian species as non-target organisms
"Considering the conclusion of ED assessment for mammalian species, the ED criteria for EATS-modality is considered not met for mammalian species as non-target organisms."

Non-target organisms other than mammals
"None of the ecotoxicological studies conducted with the active substance glyphosate was found to show EATS-mediated adversity to in birds, fish and amphibians. For EAS-mediated adversity, the effects are classified as "sensitive to, but not diagnostic of EATS" modalities and "systemic toxicity". Secondary effects were considered as a consequence of systemic toxicity.

Two studies are available that investigate EATS-related endocrine activity: an Amphibian Metamorphosis Assay for the T-modality and a Fish Short-Term Reproduction Assay for EAS-modality. Relevant parameters for T-modality and EAS-modality have been sufficiently investigated. There is no indication of EATS related endocrine activity.

Therefore, it is concluded that ED criteria with regards to non-target organisms are not met for glyphosate."

References:

EFSA. 2017. Peer review of the pesticide risk assessment of the potential endocrine disrupting properties of glyphosate. EFSA Journal. 15:4979. Accessed November 30, 2019. https://www.efsa.europa.eu/en/efsajournal/pub/4979

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Levine SL, Webb EG, Saltmiras DA. (2020) Review and analysis of the potential for glyphosate to interact with the estrogen, androgen and thyroid pathways. Pest Manag Sci. 76:2886-2906. https://onlinelibrary.wiley.com/doi/epdf/10.1002/ps.5983

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