

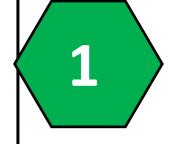


Glyphosate is Not an Endocrine Disruptor: Weight of Evidence versus Key Characteristic for Endocrine Disruption Assessment

APPLIED PHARMACOLOGY AND TOXICOLOGY, INC.

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INTRODUCTION



Regulatory authorities (US EPA 2015, EFSA 2017, Assessment Group on Glyphosate (AGG/Rapporteur Member States for EU Annex Renewal) 2022) and a peer reviewed publication (Levine et al., 2020) have used the established weight of evidence (WoE) approach and evaluated other scientifically relevant information from validated test systems and publications as the basis for their endocrine disruption (ED) assessments on glyphosate (GLY)



These WoE assessments have consistently concluded that GLY lacks ED properties due to its lack of potential to act via estrogen, androgen, thyroid or steroidogenic (EATS) modes of action (MoAs)



In contrast, a publication by Munoz et al. (2020) concluded the opposite based on a novel set of 10 Key Characteristics (KCs) of endocrine-disrupting chemicals (EDCs) developed by La Merrill et al. (2020)

• Le Merrill et al:

- · devised an approach that has not been empirically tested or validated
- derived their concept for the approach based on flawed analogies to a similar approach developed for carcinogens and ignored analyses revealing its poor performance
- mischaracterized their proposal as a consensus recommendation, when in fact, they followed no recognized method for developing a consensus document and failed to include experts who have published contrasting interpretations
- ignored fundamental principles of hormone action that have been known for more than a century and wellestablished characteristics of dose-response in endocrine pharmacology and toxicology
- · developed an approach that lacks a means to reach a negative conclusion about a chemicals' ED properties



Munoz et al. found GLY to possess 8 of the 10 KCs proposed by La Merrill et al. for ED (see red circles). To understand this striking discrepancy, we compared the WoE and the KC approach against several established regulatory evaluation criteria (US EPA, 2020, EFSA, 2017, OECD, 2019). The authors:

- omitted regulatory guideline studies including those on GLY's reproductive and developmental toxicity and endocrine disrupting potential and numerous relevant and reliable publications
- · relied on a small subset of results from non-standard and unvalidated in vitro and in vivo assays that are not traditionally used to inform regulatory safety assessments
- · did not apply, a means of determining the relevance, reliability and quality of data selected for their assessment
- did not address conflicting results
- · did not consider the basic principles of pharmacology, hormone action, and dose-response
- selected 50 publications of which almost half included studies that tested GLY-based formulations (GBF) and the results reported in these studies result from non-endocrine effects (e.g. cytotoxicity)



As examples, two tables provide a list of the publications Munoz et al. used to support their conclusion of GLY meeting the KCs of KC 1. ("current evidence indicates that glyphosate can favor hormonal receptor activity, particularly ERα by stimulating their transcriptional activation and therefore promoting phenotype changes in breast cell line models. Nevertheless, the molecular mechanism of interaction is unknown.") and for evidence that glyphosate had effects on different hormone-producing or hormone-sensitive cells as depicted in their Figure 1. Also provided in the tables are the test materials, study type, route of exposure and results/comments on the listed publications

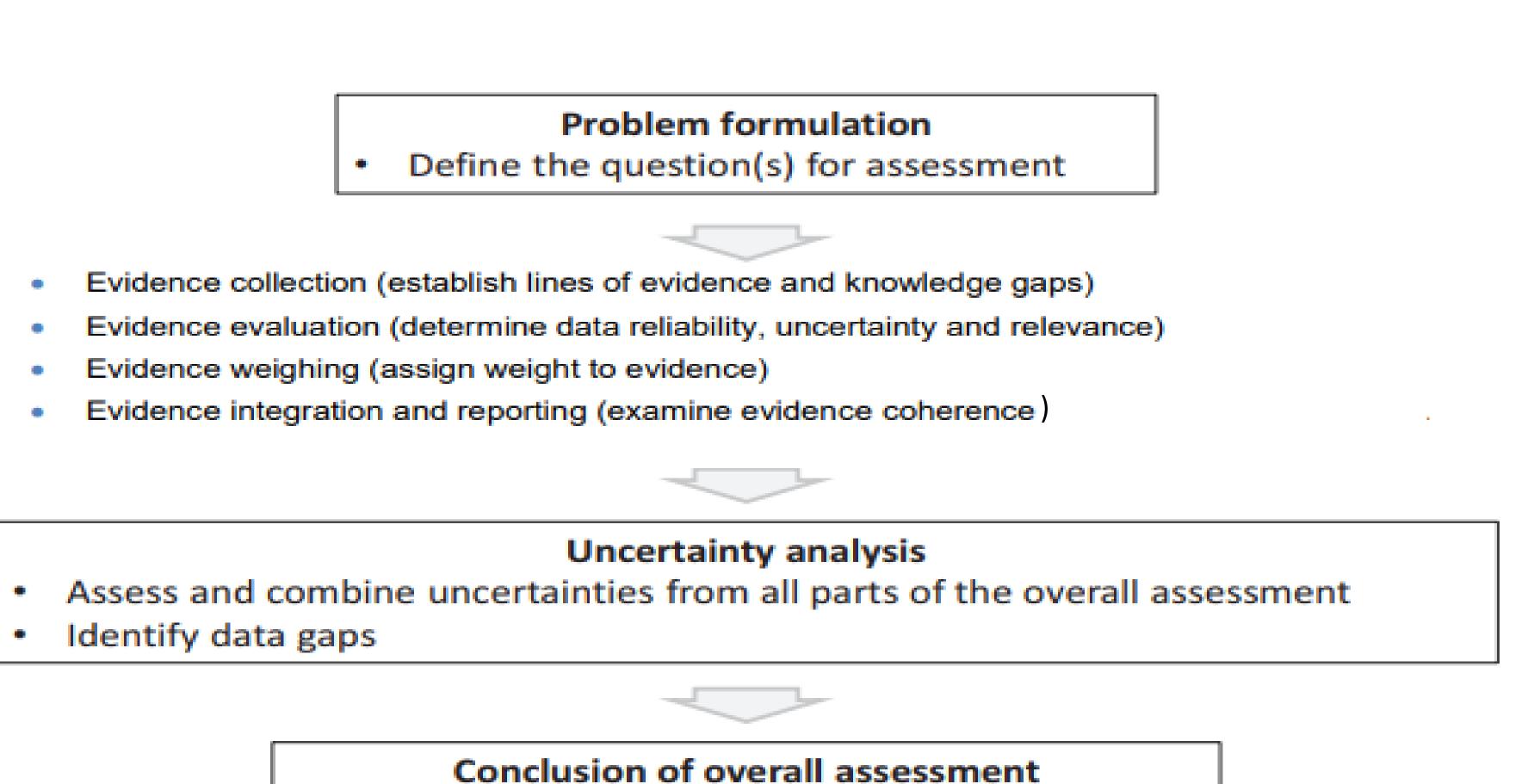


Regulatory WoE Scientific Assessment









US EDSP Tier 1 Assays Assays ER binding (Rat uterine cytosol)

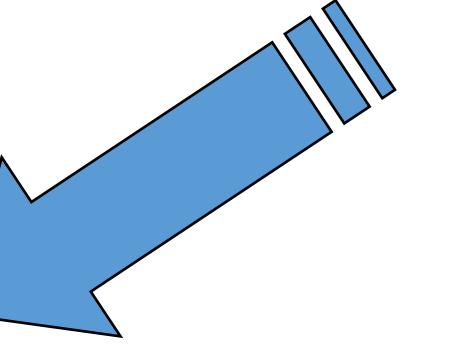
	Assays	rest Guideline
	ER binding (Rat uterine cytosol)	OSCPP 890.1250
	ERα Transcriptional Activation Assay (Human cell line HeLa 9903)	OSCPP 890.1300; OECD 455
	AR Binding Assay (Rat prostate cytosol)	OSCPP 890.1150
	Steroidogenesis Assay (Human cell line H295R)	OSCPP 890.1550; OECD 456
	Aromatase Assay (human recombinant microsomes)	OSCPP 890.1200
	Uterotrophic Assay (Rat)	OSCPP 890.1600; OECD 440
	Hershberger Assay (Rat)	OSCPP 890.1400; OECD 441
	Pubertal Female Assay (Rat)	OSCPP 890.1450
	Pubertal Male Assay (Rat)	OSCPP 890.1500
	Fish Short-term Reproduction Assay	OSCPP 890.1350; OECD 229
	Amphibian Metamorphosis Assay (Frog)	OSCPP 890.1100; OECD 231

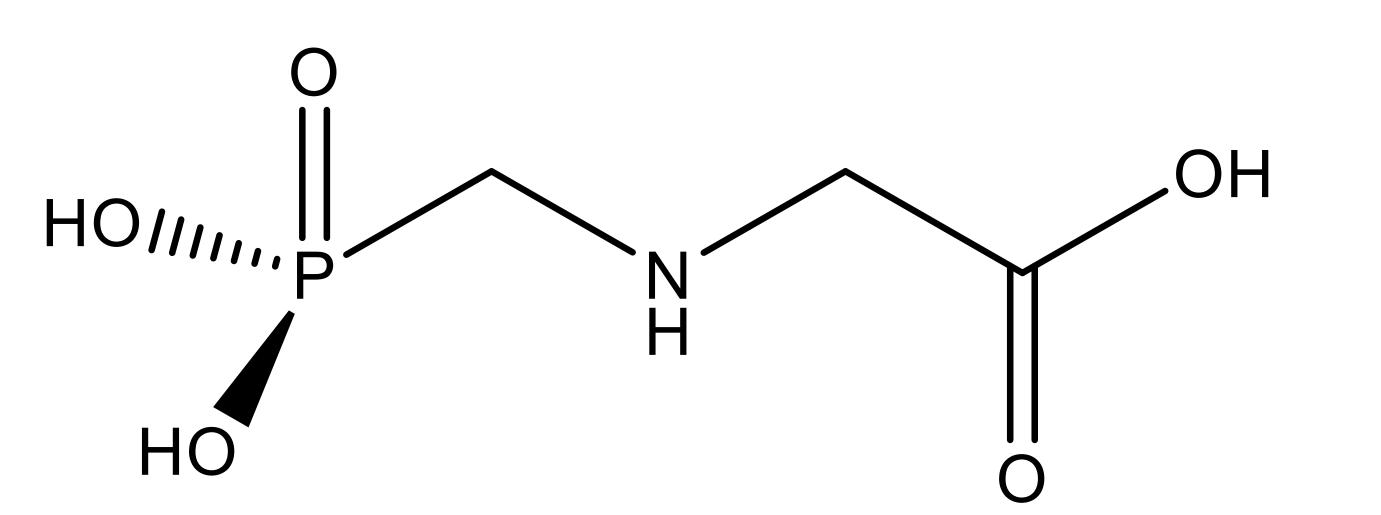
Other Scientifically Relevant Information

Study Type	Number of Studies	Test Guidelines	Endpoints
In Silico			Estrogen receptor, androgen receptor, thyroid receptor, glycocorticoid receptor, mineralocorticoid receptor, liver X receptor, peroxisome proliferator-activated receptor, retinoid X receptor, aryl hydrocarbon receptor, pregnane X receptor, and CYP3A4 activation
Subchronic Toxicity	31	OPPTS 870.3050, 870.3100, 870.3150, 870.3200	Growth, ovarian weight, adrenal weight, testes weight and histopathological evaluation of the following organs and/or systems – uterus, ovary, thyroid, adrenal, pituitary, oviduct, parathyroid, epididymides, testis, prostate and seminal vesicle
Chronic Toxicity/ Carcinogenicity	27	OPPTS 870.4200, 870.4300	Growth, ovarian weight, thyroid weight, adrenal weight, testes weight, histopathological evaluation of the following organs and/or systems - uterus, ovary, thyroid, adrenal, pituitary, epididymides, testis, prostate, seminal vesicle, and preputial gland
Developmental Toxicity	15	OPPTS 870.3700	Growth, uterine weight, pup malformations
Reproductive Toxicity	9	OPPTS 870.3800	In addition to reproductive parameters, intersex and sex ratio these endpoints are examined in animals exposed during critical developmental stages including prenatal, early postnatal and the peripubescent period into adulthood
			Females: ovarian weight, uterine weight, adrenal weight, pituitary weight, thyroid weight, growth, age at vaginal opening, time to mating, estrus, implantation, in utero development, lactation, histopathological evaluation of the following organs and/or systems – uterus, ovary, thyroid, adrenal, pituitary and oviduct
			Males: testes weight, adrenal weight, thyroid weight, pituitary weight, epididymides weight, accessory sex organ weight, growth, age at preputial separation, histopathological evaluation of the following organs and/or systems – epididymides, testes, thyroid, adrenal, pituitary, prostate and seminal vesicle
Publications	23	None	Numerous

AGG (2022) Glyphosate ED Assessment for 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."

Which Assessment Do You Trust?





Glyphosate (N-phosphonomethyl) glycine

Which Results Do You Believe?

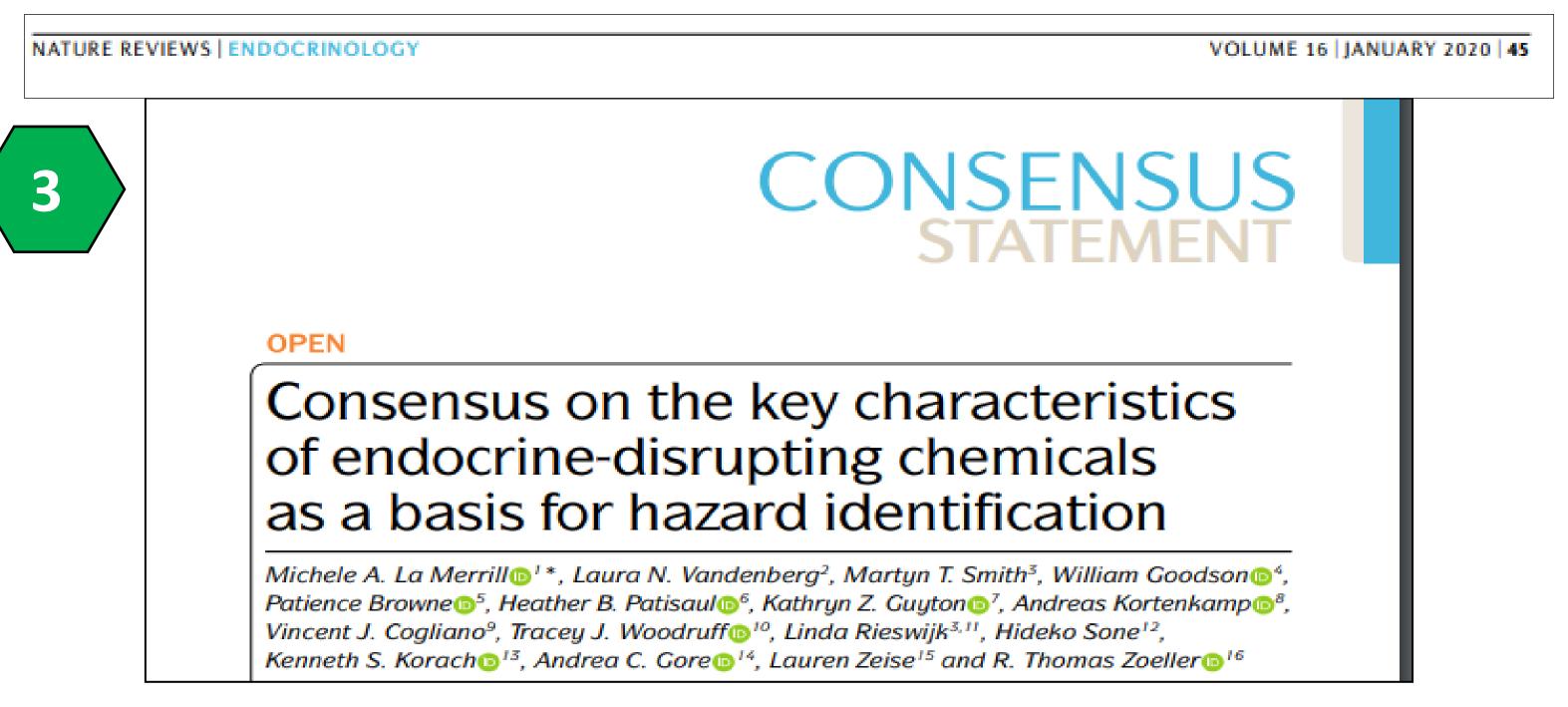
CONCLUSIONS

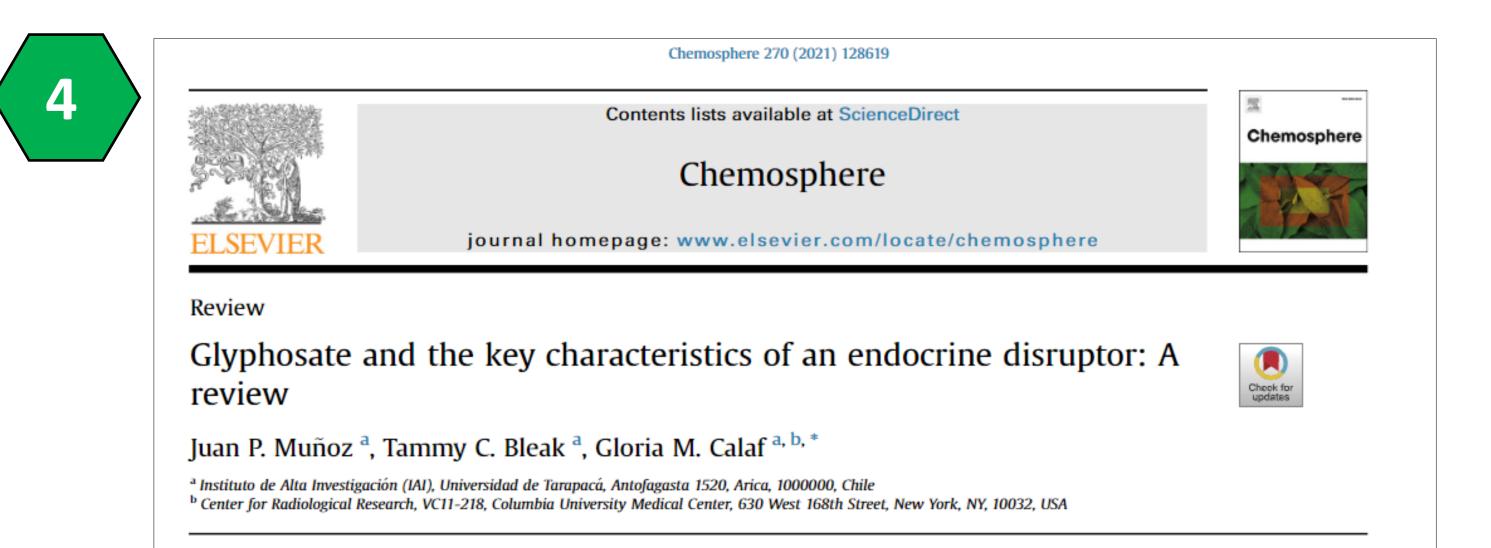
. Regulatory WoE assessments are clear that GLY does not show EATS modalities.

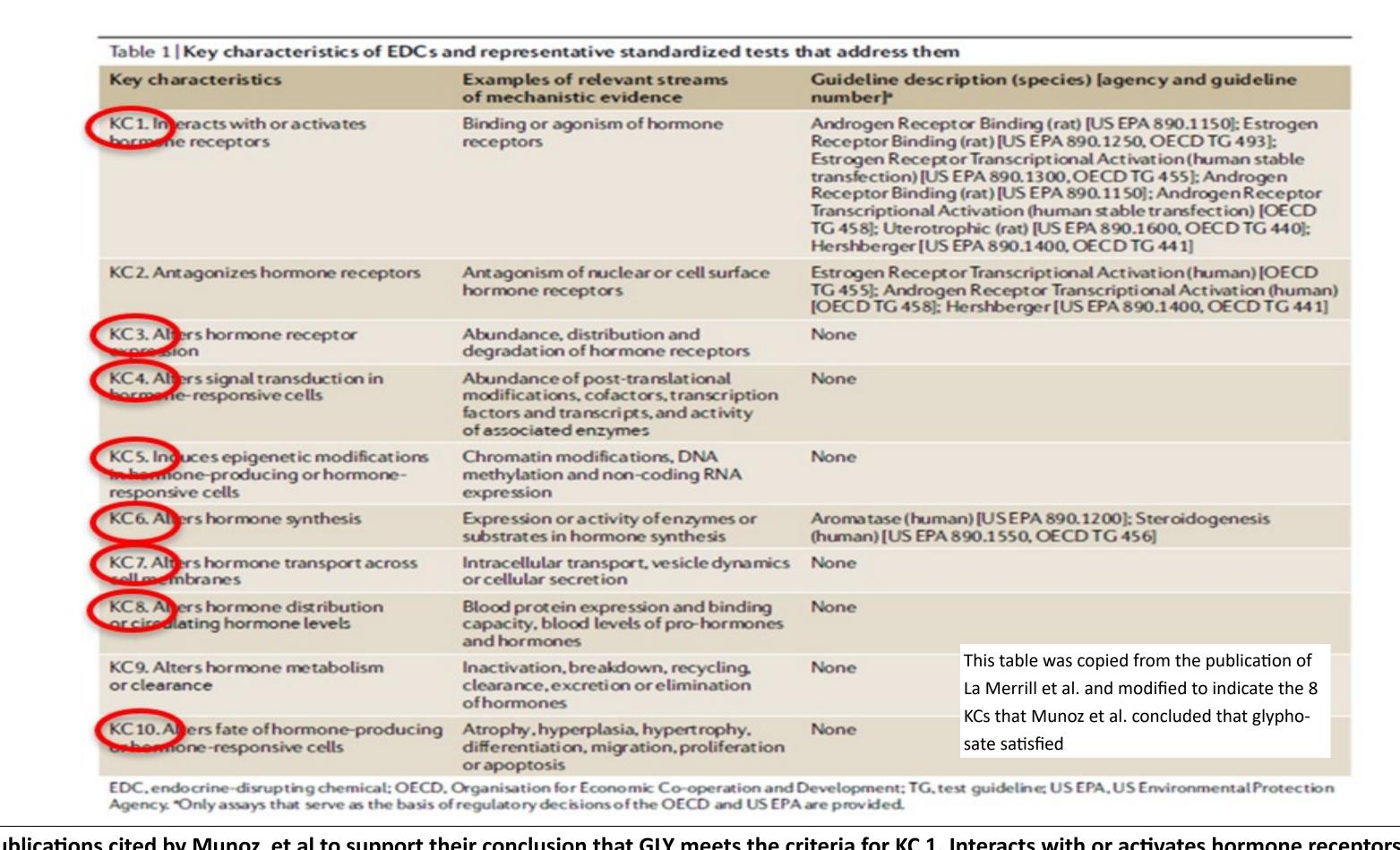
The methodological deficiencies of KCs explain the incorrect classification of glyphosate's potential as an ED using this novel approach compared to the application of well-established WoE approaches developed by regulatory agencies over the past decade.

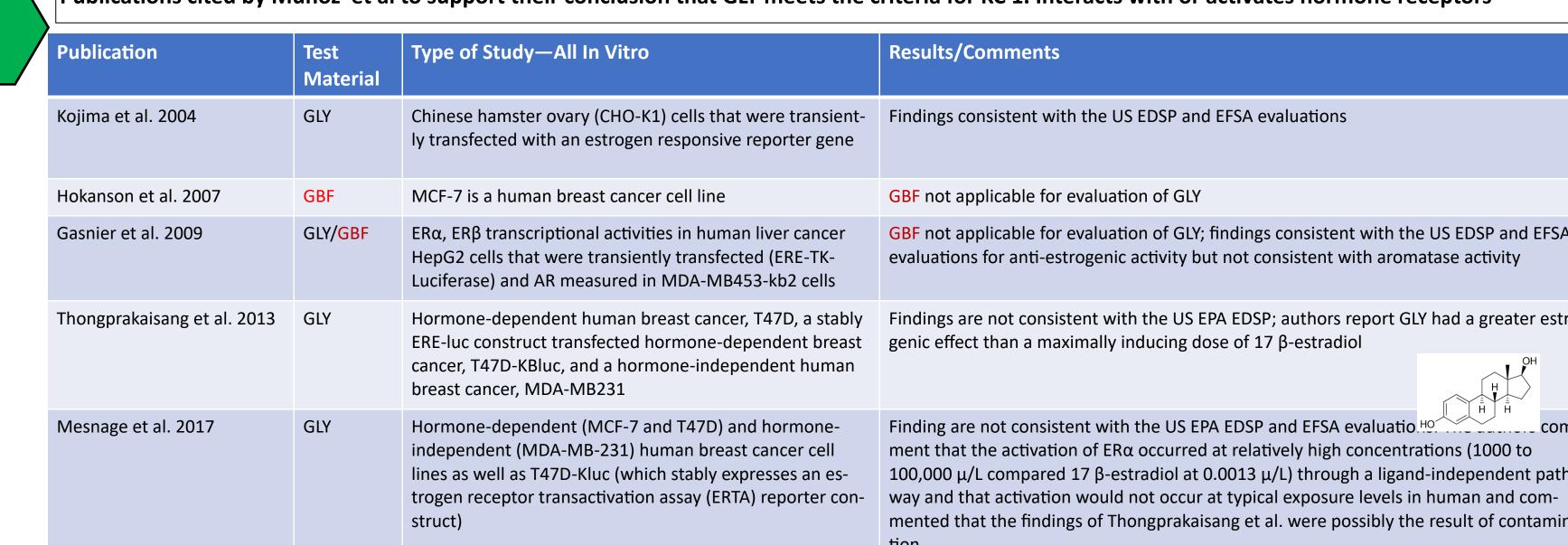
ED KCs like other KCs have not been validated against negative controls and the GLY data is an ideal example of a negative control and demonstrates how the KC approach lacks a means to get to a negative conclusion about a chemical's ED properties.

Key Characteristics









Publications cited by Munoz et al as depicted in Figure 1. of their publication as a "Summary of evidences related to the effects of glyphosate and its derivatives on different hormone-producing or hormone-sensitive cells"

Publication	Test Material	Type of Study	Route of Ex- posure	Results/Comments
Altamirano et al. 2018	GBF	In Vivo	S.C Injection	GBF not applicable for evaluation of GLY; route of exposure not relevant for human exposure
De Liz Oliveria et al. 2013	GBF	In Vitro		GBF not applicable for evaluation of GLY
De Souza et al. 2017	GBF	In Vivo	Oral Gavage	GBF not applicable for evaluation of GLY
Duforestel et al. 2019	GLY	In Vitro		Findings are inconsistent with US EDSP and EFSA evaluations
Hokanson et al. 2007	GBF	In Vitro		GBF not applicable for evaluation of GLY
Guerrero et al. 2017	GBF	In Vivo	S.C. Injection	GBF not applicable for evaluation of GLY; route of exposure not relevant for human exposure
Lorenz et al. 2019	GBF	In Vivo	Oral Dietary	GBF not applicable for evaluation of GLY
Mesnage et al. 2017	GLY/GBF	In Vitro		GBF not applicable for evaluation of GLY; GLY findings not consistent with US EDSP and EFSA evaluations
Richard et al. 2005	GLY/GBF	In Vitro		GBF not applicable for evaluation of GLY; GLY findings not consistent with US EDSP and EFSA evaluations; inhibition was observed only at concentrations of GLY that were cytotoxic
Sritana et al. 2018	GLY	In Vitro		Findings not consistent with US EDSP and EFSA evaluations; possible estrogenic contamination
Stur et al 2019	GBF	In Vitro		GBF not applicable for evaluation of GLY
Thongprakaisang et al. 2013	GLY	In Vitro		Findings not consistent with US EDSP and EFSA evaluations; possible estrogenic contamination
Walsh et al. 2000	GLY/GBF	In Vitro		GBF not applicable for evaluation of GLY; GLY findings were consistent with US EDSP and EFSA evaluations