UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

MEMORANDUM

Date: January 13, 2019

SUBJECT: Glyphosate: Response to Comments on the Proposed Interim Decision Regarding the Human Health Risk Assessment

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40 CFR: §180.364

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The Office of Pesticide Programs received hundreds of thousands of public comments on the proposed interim decision (PID) for glyphosate as part of registration review. Comments regarding the human health risk assessment came from a wide array of stakeholders. Topics relating to human health included concerns with the agency's cancer assessment, toxicological studies, protection of children, and detections of glyphosate. These comments regarding the human health risk assessment for glyphosate have been previously addressed in the *Glyphosate: Response to Comments on the Human Health Draft Risk Assessment* (D448021; M. Perron; 23-APR-2018) and did not result in changes to the agency's risk assessment.

During the public comment period, 65 open literature studies were also identified for the agency's consideration (Appendix A). Of these, 23 were previously identified and considered by

the agency as part of two open literature searches conducted to support the draft human health risk assessment for registration review. The agency reviewed the open literature as part of the *Glyphosate Systematic Review of Open Literature* (D417703; TXR 0056885; M. Perron; 12-DEC-2017) for hazard identification and characterization purposes in order to identify studies that could potentially impact the human health risk assessment. A fit-for-purpose systematic review was also executed to obtain relevant and appropriate open literature studies with the potential to inform the human carcinogenic potential of glyphosate and detailed in the *Revised Glyphosate Issue Paper: Evaluation of Carcinogenic Potential* (D444689; TXR 0057688; G. Akerman; 12-DEC-2017).

The remaining 42 studies identified during the public comment period were primarily journal articles published since these searches were conducted. The majority of the studies did not warrant detailed evaluation for a variety of reasons, such as no effects seen from glyphosate exposure, administration via a non-relevant route, effects seen at doses higher than the current points of departure, only one dose was tested eliminating the potential for dose-response evaluation, lack of glyphosate measurements, and exposure or biomonitoring studies that would not impact risk estimates. Furthermore, several studies were conducted *in vitro* or evaluated biochemical or molecular effects that are difficult to translate into *in vivo* effects. These studies are typically considered as part of mode of action/adverse outcome pathway (MOA/AOP) analyses. As a result, *in vivo* studies are given more weight at this time.

For the *in vivo* studies identified, the most common limitations/deficiencies were related to the nature of the test substance(s) used for exposure. Many of the studies used commercial formulations or dilutions; however, direct measurements of the active ingredient were not conducted in order to determine actual dose concentrations and/or identification information was not provided for the formulation used. There are numerous glyphosate formulations and providing a general product name, such as Roundup, does not provide the agency with information to ascertain the exact formulation used and determine all of its chemical components. Additionally, several studies were conducted in other countries and utilized formulations that are not registered in the United States. As a result, the active ingredients and other components of the formulation are unknown, and any potential effects cannot be attributed to glyphosate and/or defined glyphosate concentrations.

Several *in vitro* genotoxicity studies were identified for consideration. Although positive results were observed in some of these studies, there would be no impact on the agency's weight of evidence evaluation of the genotoxic potential of glyphosate since there is sufficient evidence in the existing database (described in the *Revised Glyphosate Issue Paper: Evaluation of Carcinogenic Potential*; D444689; TXR 0057688; G. Akerman; 12-DEC-2017) to conclude that the *in vitro* effects claimed by the study authors do not lead to genotoxicity *in vivo*.

Two additional journal articles on the association between glyphosate exposure and non-Hodgkin lymphoma (NHL) were identified for detailed review (D455531; D. Miller; 6-JAN-2020;). The following are brief summaries of these journal articles and the agency's reviews:

- Zhang et al. (2019) is a review article summarizing epidemiological studies between 2001 and 2018 on the association between glyphosate exposure and NHL. All of the data/information included in the article was previously considered by the agency as part

of the *Revised Glyphosate Issue Paper: Evaluation of Carcinogenic Potential* (D444689; TXR 0057688; G. Akerman; 12-DEC-2017); however, the authors conducted their own meta-analysis to incorporate recently published risk estimates for the Agricultural Health Study (AHS) cohort (Andreotti et al., 2018) based on their *a priori* hypothesis that the highest exposures to glyphosate will lead to increased risk of NHL in humans[1]. In its detailed review of this study, the agency identified concerns with the meta-analysis as performed by the authors. A supplemental analysis by the agency indicated that a lower non-statistically significant meta-risk ratio of 1.14 (95% CI: 0.87-1.50) would be obtained if the Andreotti et al. (2018) study is properly incorporated into the meta-analysis[2]. Therefore, the meta-analyses performed by Zhang et al. (2019) would not impact the conclusions presented in the agency's revised issue paper.

- Leon et al. (2019) is a pooled analysis of NHL in agricultural cohorts from France, Norway, and the United States (the AHS cohort). For overall NHL malignancies and NHL subtypes, except diffuse large B-cell lymphoma (DLBCL), glyphosate risk estimates were less than 1. For DLBCL a somewhat elevated, but non-statistically significant risk estimate of 1.36 (95% CI: 1.00-1.85) was observed. While the analysis benefited from a combined cohort of more than 300,000 farmers and farmworkers from different countries, only the cohort from the United States used actual measurement instruments (self-administered questionnaire) for glyphosate exposure where a smaller risk estimate for the DLBCL subtype of 1.2 (95% CI: 0.72-1.98) was obtained. Furthermore, the nature and characteristics of the three cohorts differed in substantive ways and it is not clear that the statistical adjustments made were adequate to account for these differences.

Therefore, none of the open literature studies identified for the agency's consideration were found to have an impact on the glyphosate hazard characterization, cancer assessment, or human health risk assessment. The agency will continue to monitor the open literature for studies that use scientifically sound and appropriate methodology and relevant routes of exposure that have the potential to impact the risk evaluation of glyphosate.

[1] Here, higher exposures correspond to higher levels, longer durations, and/or greater lags/longer latencies.
[2] We note, however, that the meta-estimate in our Revised Glyphosate Issue paper was 1.27 (95% CI:1.01, 1.59) while our updated estimate after incorporating the Andreotti et al. (2018) paper is 1.14 (95% CI: 0.87, 1.50). See D. Miller; D455531; 6-JAN-2020 for details and information.

Appendix A. Studies identified for the agency's consideration during the public comment period for the Proposed Interim Decision (PID).

Author	Year	Title	Part of previous agency open literature reviews?	Comments
Aiassa, D	2019	Evaluation of genetic damage in pesticide applicators from the province of Cordoba, Argentina	No	ecological epidemiological study; no glyphosate-specific estimates; would be assigned low quality ranking according to process detailed in revised issue paper on evaluation of carcinogenic potential
Alvarez-Moya, C	2013	Comparison of the *in vivo* and *in vitro* genotoxicity of glyphosate isopropylamine salt in three different organisms	Yes	--
Astiz, M	2009	Antioxidant defense system in rats simultaneously intoxicated with agrochemicals	Yes	--
Baier, Carlos J	2017	Behavioral impairments following repeated intranasal glyphosate-based herbicide administration in mice	No	Argentina formulation; micropipette administration into nostrils (not a relevant route); one dose tested; no glyphosate measurements
Benbrook, CM	2019	How did US EPA and IARC reach diametrically opposed conclusions on the genotoxicity of glyphosate-based herbicides?	No	review paper; examined list of studies published since agency's evaluation; supplemental tables comparing EPA and IARC yielded one study to reexamine (Gasnier et al. 2009 included in table below)
Bolognesi, C	2009	Biomonitoring of genotoxic risk in agricultural workers from five Colombian regions: association to occupational exposure to glyphosate	Yes	--
Bolognesi, C	1997	Genotoxic Activity of Glyphosate and Its Technical Formulation Roundup	Yes	--

Author	Year	Title	Part of previous agency open literature reviews?	Comments
Bolognesi, C	2011	Micronuclei and pesticide exposure	No	Review article for biomonitoring studies for several pesticides; already considered the one study on glyphosate (Bolognesi et al. 2009) in previous systematic review
Brendler-Schwaab, S	2005	The *in vivo* comet assay: use and status in genotoxicity testing	No	No glyphosate information
Caballero, M	2018	Estimated Residential exposure of agricultural chemicals and premature mortality by Parkinson's disease in Washington state	No	Glyphosate exposure assessed indirectly using geospatial information on residential address and crop data from WA State from 2011-2015; residential address was based only on address listed in the death registry for 2011-2015 so the analysis was cross-sectional in nature and was unable to assess potential lifetime exposure based on either changes in WA State agriculture or changes in residential address; statistical analysis only considered the demographic variables sex, race, marital status, and education indicating limited ability to assess confounding or control for co-exposure to pesticides and other environmental factors; given these limitations, the modest risk estimate of 1.3 (95% CI: 1.0-1.62) may reflect residual confounding and is based on a low quality exposure assessment that did not directly assess individual exposure or allow the investigators to assess lifetime exposure

Author	Year	Title	Part of previous agency open literature reviews?	Comments
Cavalli, VL	2013	Roundup disrupts male reproductive functions by triggering calcium-mediated cell death in rat testis and Sertoli cells	Yes	--
Dimitroy, BD	2006	Comparative genotoxicity of the herbicides Roundup, Stomp and Reglone in plant and mammalian test systems	Yes	--
Gasnier, C	2009	Glyphosate-based herbicides are toxic and endocrine disruptors in human cell lines.	Yes	In previous open literature review considered non-relevant; reexamination indicated it is relevant; in vitro study evaluating glyphosate alone and formulations for cytotoxicity, estrogenic activity, anti-androgenic activity and aromatase disruption; only effect reported for glyphosate alone was a statistically significant, non-concentration dependent increase in anti-androgenic activity
Gehin, A	2005	Vitamins C and E reverse effect of herbicide-induced toxicity on human epidermal cells HaCat: a biochemometric approach	Yes	--
Ghisi, N	2016	Does exposure to glyphosate lead to an increase in the micronuclei frequency? A systematic and meta-analytic review	Yes	--
Gillezeau, C	2019	The evidence of human exposure to glyphosate: a review	No	Exposure study; review article; no impact on risk estimates
Grisolia, C	2002	A comparison between mouse and fish micronucleus test using cyclophosphamide, mitomycin C and various pesticides	Yes	--
Heu, C	2012	Glyphosate-induced stiffening of HaCaT keratinocytes, a Peak Force Tapping study on living cells	Yes	--

Author	Year	Title	Part of previous agency open literature reviews?	Comments
Jiang, Xiao	2018	A commercial Roundup formulation induced male germ cell apoptosis by promoting the expression of XAF1 in adult mice.	No	Formulation by gavage at 60, 180 or 540 mg/kg/day and in vitro exposures; no glyphosate measurements; potential effects only seen at dose higher than current POD
Kasuba, V	2017	Effects of low doses of glyphosate on DNA damage, cell proliferation and oxidative stress in the HepG2 cell line	No	In vitro study evaluating DNA damage, cell proliferation and oxidative stress in HepG2 cells tested at 3 concentrations of glyphosate; non-statistically significant increase in proliferative response; primary DNA damage (comet assay) decreased relative to negative control; increase in MN and effects on lipid peroxidation were not concentration dependent; no convincing glyphosate-related effects
Kojima, H	2010	Endocrine-disrupting Potential of Pesticides via Receptors and Aryl Hydrocarbon Receptor	No	Mini-review; only mention of glyphosate is in one table and it was negative for all receptors
Kongtip, P	2017	Glyphosate, and paraquat in maternal and fetal serum in Thai Women	No	Exposure study; no impact on risk estimates
Kubsad, D	2019	Assessment of Glyphosate Induced Epigenetic Transgenerational Inheritance of Pathologies and Sperm Epimutations: Generational Toxicology	No	Intraperitoneal injection of glyphosate at 25 mg/kg/day in pregnant females; not a relevant route
Kwiatkowska, M	2016	DNA damage and methylation induced by glyphosate in human peripheral blood mononuclear cells (in vitro study)	No	Report primary DNA damage at high concentrations of glyphosate (0.5 mM and higher) and increase in DNA methylation of p53 and p16 promoters; methylation was statistically significantly increased at both concentrations for the p53 promoter, but not p16; not enough concentrations tested to make a conclusion on impact of glyphosate on p53 methylation.

Author	Year	Title	Part of previous agency open literature reviews?	Comments
Leon, M	2019	Pesticide use and risk of non-Hodgkin lymphoid malignancies in agricultural cohorts from France, Norway and the USA: a pooled analysis from the AGRICOH consortium	No	Detailed review performed (D. Miller; 6-JAN-2020; D455531); summarized in text above
Lioi, M	1998	Genotoxicity and oxidative stress induced by pesticide exposure in bovine lymphocyte cultures in vitro	Yes	--
Lueken, A	2004	Synergistic DNA damage by oxidate stress (induced by H2O2) and nongenotoxic environmental chemicals in human fibroblasts	Yes	--
Manas, F	2009	Genotoxicity of AMPA, the environmental metabolite of glyphosate, assessed by the Comet assay and cytogenetics test	Yes	--
Manas, F	2009	Genotoxicity of glyphosate by the comet assay and cytogenetic test	Yes	--
Manservisi, F	2019	The Ramazzini Institute 13- week pilot study glyphosate-based herbicides administered at human-equivalent dose to Sprague Dawley rats: effects on development and endocrine system	No	Glyphosate and formulation; no blinding to dose; small sample size for developmental evaluation (n=8/dose); generally no adverse effects observed; only one dose tested; no glyphosate measurement
Mao, Q	2018	The Ramazzini Institute 13-week pilot study glyphosate-based herbicides administered at human-equivalent dose to Sprague Dawley rats: effects on the microbiome	No	Glyphosate and formulation; no glyphosate measurement; evaluated changes in microbiome; no link to adverse apical outcomes
Martinez, A	2019	Effects of glyphosate and aminomethylphosphonic acid on an isogeneic model of the human blood-brain barrier	No	In vitro study; no glyphosate measurements
Mensah, PK	2015	Ecotoxicology of glyphosate and glyphosate-based herbicide - toxicity to wildlife and humans	No	Book chapter; all references on human health effects from glyphosate already considered

Author	Year	Title	Part of previous agency open literature reviews?	Comments
Mesnage, R	2016	Multiomics reveal non-alcoholic fatty liver disease in rats following chronic exposure to an ultra-low dose of Roundup herbicide	No	Belgium formulation; proteome and metabolome data; changes may reflect adaptive liver effects; no link to adverse apical outcomes
Milesi, MM	2018	Perinatal exposure to a glyphosate-based herbicide impairs female reproductive outcomes and induces second-generation adverse effects in Wistar rats	No	Argentina formulation by diet at 2 or 200 mg/kg/day; potential effects at dose higher than current POD
Milic, M	2018	Oxidative stress, cholinesterase activity, and DNA damage in the liver, whole blood, and plasma of Wistar rats following a 28-day exposure to glyphosate	No	Glyphosate administered via gavage at 0.1, 0.5, 1.75, and 10 mg/kg/day; glyphosate was not measured in dose preparations and small sample size (n=5) used; no change in body and liver weights; plasma and liver ROS and plasma GSH levels similar to controls, no change in GSH activity in blood, inconsistent cholinesterase data and no neurotoxicity MOA/AOP for glyphosate
Mills, PJ	2019	Glyphosate excretion is associated with steatohepatitis and advanced liver fibrosis in patients with fatty liver disease	No	Study measured glyphosate/AMPA metabolite excretion levels in NAFLD patients who were either NASH (Non-Alcoholic Steatohepatitis) or not NASH patients and compared glyphosate and AMPA measurement in urine; no subjects without NAFLD; problems with temporality; single urine measurement only; no information collected on dietary intake or occupation
Mills, PJ	2017	Excretion of the herbicide Glyphosate in older adults between 1993-2016	No	Exposure/biomonitoring study; no impact on risk estimates
Niemann, L	2015	A critical review of glyphosate findings in human urine sample and comparison with the exposure of operators and consumers	No	Review article for exposure; no impact on risk estimates; calculations show glyphosate exposure well below

Author	Year	Title	Part of previous agency open literature reviews?	Comments
				acceptable daily intake (ADI) and acceptable operator exposure levels (AOEL)
Parvez, S	2018	Glyphosate exposure in pregnancy and shortened gestational length: a prospective Indiana birth cohort study	No	Glyphosate levels in urine and drinking water; cross-sectional study evaluating correlation; no AMPA metabolite measured; small sample size; limited diversity; no evaluation of other chemical exposures (except smoking, caffeine, and alcohol)
Panzacchi, S	2018	The Ramazzini Institute 13-week pilot study glyphosate-based herbicides administered at human-equivalent dose to Sprague Dawley rats: study design and first in-life endpoints evaluation	No	Glyphosate and formulation; no glyphosate measurement; no adverse effects observed
Perry, M	2019	Historical evidence of glyphosate exposure from a US agricultural cohort	No	Exposure/biomonitoring; no impact on risk estimates
Pham, T	2019	Perinatal exposure to glyphosate and a glyphosate-based herbicide affect spermatogenesis in mice	No	Glyphosate and Belgium formulation; no glyphosate measurement; small sample size for some parameters; no adverse effect in several parameters; small magnitude of change and/or lack of dose response in others; no incidence or severity scores reported for histopathological evaluations
Portier, C	2016	Difference in the carcinogenic evaluation of glyphosate between the IARC and EFSA	Yes	--
Prasad, S	2009	Clastogenic Effects of Glyphosate in Bone Marrow Cells of Swiss Albino Mice	Yes	--
Richard, S	2005	Differential Effects of Glyphosate and Roundup on Human Placenta Cells and Aromatase	Yes	--

Author	Year	Title	Part of previous agency open literature reviews?	Comments
Roustan, A	2014	Genotoxicity of mixtures of glyphosate and atrazine and their environmental transformation products before and after photoactivation	Yes	--
Saleh, SM	2018	Hepato-morphology and biochemical studies on the liver of albino rats after exposure to glyphosate-Roundup	No	Egypt formulation; no glyphosate measurements; histopathological findings cannot be interpreted without incidence or severity scoring
Santovito, A	2018	In vitro evaluation of genomic damage induced by glyphosate on human lymphocytes	No	Authors report chromosomal aberrations and micronuclei formation in human lymphocytes treated in vitro with 0.025 µg/mL glyphosate and above; effects reported are inconsistent with other findings in the literature; sample size too small to make reliable conclusions for low test concentration
Schimpf, MG	2015	Neonatal exposure to a glyphosate based herbicide alters the development of the rat uterus	No	sc injection (not a relevant route); formulation not registered in US; no glyphosate measurements; only one dose tested
Sena de Souza, J	2019	Maternal glyphosate-based herbicide exposure alters antioxidant-related genes in the brain and serum metabolites of male rat offspring	No	Brazil formulation; gene expression profiles; no link to adverse apical outcomes
Sena de Souza, J	2017	Perinatal exposure to glyphosate-based herbicide alters the thyrotrophic axis and causes thyroid hormone homeostasis imbalance in male rats	No	Brazil formulation; no glyphosate measurements; no changes in T3 or T4; TSH decreased rather than increased and presented a flat dose response; hormone measurement calibration information not reported; gene and metabolomic data
Seneff, S	2018	Is Glyphosate a key factor in mesoamerican nephropathy?	No	Review article; hypothesis generating paper
Sivikova, K	2005	Cytogenetic effect of technical glyphosate on cultivated bovine peripheral lymphocytes	Yes	--

Author	Year	Title	Part of previous agency open literature reviews?	Comments
Stur, E	2019	Glyphosate-based herbicides at low doses affect canonical pathways in estrogen positive and negative breast cancer cell lines	No	Brazil formulation testing with breast cancer cell lines; no glyphosate measurements
Szepanowksi, F	2018	Differential impact of pure glyphosate and glyphosate-based herbicide in a model of peripheral nervous system myelination	No	No differences in myelination in cultures treated with glyphosate compared to vehicle control
Teleken, JL	2019	Glyphosate-based herbicide exposure during pregnancy and lactation malprograms the male reproductive morphofunction in F1 offspring	No	Brazil formulation; no glyphosate measurements/only one dose tested that is above current POD
Thongprakaisang, S	2013	Glyphosate induces human breast cancer cells growth via estrogen receptors	Yes	--
Townsend, M	2017	Evaluation of various glyphosate concentrations on DNA damage in human Raji cells and its impact on cytotoxicity	No	DNA damage in vitro only at concentrations that the authors report as several orders of magnitude larger than those attainable in vivo
Vigfusson, N	1980	The effect of the pesticides, Dexon, Captan and Roundup, on sister-chromatid exchanges in human lymphocytes in vitro	Yes	--
Von Ehrenstein, O	2019	Prenatal and infant exposure to ambient pesticides and autism spectrum disorder in children: population based case-control study	No	Assumed exposure from agricultural application records (no actual measurements) other exposure pathways not considered; addresses at time of enrollment may not reflect an individual's exposure over pregnancy
Walsh, L	2000	Roundup Inhibits Steroidogenesis by Disrupting Steroidogenic Acute Regulatory (StAR) Protein Expression	Yes	--
Wang, L	2019	Glyphosate induces benign monoclonal gammopathy and promotes multiple myeloma progression in mice	No	incorrect EPA chronic reference dose cited; only one dose tested that is above current POD

Author	Year	Title	Part of previous agency open literature reviews?	Comments
Wozniak, E	2018	The mechanism of DNA damage induced by Roundup 360 PLUS, glyphosate and AMPA in human peripheral blood mononuclear cells - genotoxic risk assessment (Accepted Manuscript)	No	In vitro study using human lymphocytes; DNA damage at glyphosate concentrations of 250 µM and higher
Zhang, L	2019	Exposure to glyphosphate-based herbicides and risk for non-Hodgkin lymphoma: A meta-analysis and supporting evidence	No	Detailed review performed (D. Miller; 6-JAN-2020; D455531); summarized in text above