EXHIBIT 1

Expert Report
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Executive Summary

I. Background and Expertise

I received my Ph.D in biostatistics from the University of North Carolina – Chapel Hill in 1981. Most of my research has focused on the design, analysis, and interpretation of environmental health data with a focus on carcinogenicity. I am currently a Senior Collaborating Scientist (part-time) with the Environmental Defense Fund, and an Adjunct Professor at Emory University and Maastricht University. I am also working with several governments on risk assessment issues and as a consultant on chemical-related issues (including glyphosate) to several law firms.

I have authored more than 200 peer-reviewed publications and book chapters. During my 36+ years of research, I have focused on using systems-based approaches to understand the impact of the environment on human health. I have received numerous awards including the President's Dream Green Team Award from President Obama, the Spiegelman Award from the American Public Health Association and the Outstanding Practitioner of the Year Award from the International Society for Risk Analysis. I am an elected Fellow of the International Statistics Institute, the World Innovation Foundation, the American Statistical Association and the Collegium Ramazzini.

Prior to my retirement, I served as the Director of the US National Center for Environmental Health at the Centers for Disease Control and Prevention and Director of the Agency for Toxic Substances and Disease Registry (ATSDR). Prior to this, I was at the US National Institute of Environmental Health Sciences (NIEHS) where I conducted research on environmental health and served as the Director of the Environmental Toxicology Program, the Associate Director of the National Toxicology Program, and the Senior Scientific Advisor to the Director of NIEHS.

Throughout my career, I have participated in or directed risk assessments of environmental agents. Through the International Agency for Research on Cancer (IARC), I have participated in 8 reviews of chemicals to see if they cause cancer, including glyphosate. Through the World Health Organization (WHO), I have participated in the development of two risk assessments (dioxins and DDT). While a member and Chairperson of the USEPA FIFRA Scientific Advisory Panel, I have participated in the

review of dozens of EPA risk assessments of pesticides. In association with EPA's Scientific Advisory Board, I have helped them to develop their Carcinogen Risk Assessment Guidelines. I have also helped the IARC develop their guidelines as well. While at the NIEHS, I was in charge of the Report on Carcinogens (ROC), the US Department of Health and Human Services official list of chemicals and agents that are likely to cause cancer in humans. While Director of the ATSDR, I was in charge of their Tox Profiles Program, which does risk assessments for chemicals that are found in superfund sites in the US. While in charge of these two programs (ROC and Tox Profiles), I had both programs reviewed and changed their methodology to match current approaches.

More detail on my qualifications and expertise is provided in Chapter 2 in the full report.

II. Bradford Hill Evaluation and Data Summaries

Most of the guidelines [1-3] used for cancer risk assessment trace their origins to a paper by Hill (1965) [4]. The IARC review of glyphosate [5] followed guidelines derived from Hill (1965) and concluded glyphosate was "probably carcinogenic to humans".

The overall goal of this Expert Report is to determine the degree to which the available scientific evidence supports a causal linkage between exposure to glyphosate and non-Hodgkin's lymphoma (NHL) in people. **Hill (1965)** [4] listed nine (9) aspects of epidemiological studies and the related science that one should consider in assessing causality. The presence or absence of any of these aspects is neither sufficient nor necessary for drawing inferences of causality. Instead, the nine aspects serve as means to answer the question of whether other explanations are more credible than a causal inference. The nine aspects are listed below:

An inference of causality is strengthened when several of the studies show a **consistent positive association** between cancer and the exposure.

An inference of causality is strengthened when the **strength of the observed association** in several studies are large and precise.

An inference of causality is strengthened when there is data supporting **biological plausibility** demonstrated through experimental evidence.

An inference of causality is strengthened when there is a **biological gradient** showing a reasonable pattern of changing risk with changes in exposure (e.g. risk increases with increasing exposure or with longer exposure).

An inference of causality is strengthened when there is a **temporal relationship** in which the exposure comes before the cancer.

An inference of causality is strengthened when the exposure is **specific** for a given cancer.

An inference of causality is strengthened when other lines of experimental evidence are **coherent** with a causal interpretation of the association seen in the epidemiological

evidence.

Finally, an inference of causality is strengthened when there are other chemical agents with **analogous** structures showing similar effects in humans and/or animals and/or showing similar biological impacts in mechanistic studies.

A more detailed description of these nine aspects and how they relate to the evidence that glyphosate causes NHL in humans is provided in Chapter 3. In the overall evaluation of the data on glyphosate, I will be evaluating each of these aspects to arrive at an overall conclusion.

The International Agency for Research on Cancer (IARC) preamble[6] guides Working Groups on how to evaluate scientific literature to determine if something is a hazard and is derived from the principles provided by Hill (1965) [4]. All Working Groups follow these guidelines and this process is accepted worldwide as a proper way to evaluate the literature for a hazard (e.g., the European Chemical Agency cites the IARC review process as guidance and then uses the exact same wording as IARC does to guide their own hazard evaluation process[1]).

In March 2015, the IARC (an agency of the World Health Organization) brought together seventeen scientists (the Working Group) to evaluate the scientific evidence on whether glyphosate can cause cancer in humans. The Working Group concluded that glyphosate falls in the category "probably carcinogenic to humans (Group 2A)"[5]. Their review is given in detail in Chapter 17.

III. Human Epidemiological Studies

There are seven epidemiology studies done on two different continents by different research groups using different designs, questionnaires and study populations that show consistent positive responses with no obvious bias or confounding that would explain the results. There are two additional studies that have problems with exposure misclassification and differ from the other seven in response.

A systematic literature search of all the scientific literature was used to identify all epidemiological studies that were pertinent to evaluating an association between glyphosate and NHL. The search algorithm was copied from Chang and Delzell (2016) [7] and the results are provided in Section 4.1. A total of 17 epidemiological studies were identified for this review. These studies can be divided into 14 case-controls studies (Section 4.2) and 3 cohort studies (Section 4.3).

III.a Case-Control Studies

A case-control study is an epidemiological study in which you have two groups differing in disease status (e.g. cases have NHL and controls do not) that are then evaluated to determine if they differ on some possibly causal factor (e.g. exposure to glyphosate). In case-control studies for environmental issues, it is typical to ascertain exposures

through the use of questionnaires. Recall bias[1] is a common concern with studies of this type and occurs when cases are more likely to say they are exposed to glyphosate than controls or when controls are more likely to say they are exposed to glyphosate than cases. The recall must be different for the cases than the controls for this to cause a bias; errors in recalling past exposures that happen for both cases and controls would not be recall bias. Many of the individual case-control studies checked for recall bias by obtaining pesticide purchase data from suppliers and comparing it to reported use and saw few differences between the measures suggesting little impact of recall bias in these studies.

Three case-control studies were conducted in the United States between 1976 and 1986 ([8-10]) looking at exposures to pesticides and agricultural herbicides in NHL patients. **Cantor et al. (1992)** [8] in Iowa and Minnesota, **Zahm et al. (1990)** [10] in Nebraska and **Hoar et al. (1986)** [9] in Kansas collected information on pesticide and herbicide use from NHL patients. **De Roos et al. (2003)** [11] pooled the data from these three studies to examine pesticide exposure to glyphosate in farming as risk factors for NHL. The odds ratio[2] (OR) and 95% confidence interval for any glyphosate exposure was 2.1 (1.1-4.0) in a logistic regression analysis controlling for all 47 other pesticides reported, age and study site. The authors also analyzed the data using a Bayesian hierarchical regression analysis yielding an OR (95% confidence interval) of 1.6 (0.9-2.8) controlling for the same parameters as the logistic regression. The authors note that the positive results seen in their study are not likely due to recall bias since there were few associations seen over the 47 pesticides they studied and because of additional studies comparing supplier records to recall by cases and controls. Adjustment for 47 pesticides is likely to have reduced the significance of the observed ORs for pesticides that are associated with NHL.

Nordstrom et al. (1998)[12] conducted a population-based case-control study of hairy cell leukemia (HCL), a subtype of B-cell NHL, in Sweden that included an evaluation of exposures to glyphosate. **Hardell and Eriksson (1999)**[13] conducted a population-based case-control study of all male patients older than 25 years diagnosed with NHL between 1987 and 1990 in the four most northern counties of Sweden. (0.6-54). **Hardell et al. (2002)**[14] conducted a pooled analysis of NHL and HCL by combining these two studies. The analysis controlling for age, study, county and vital status yielded an OR of 3.04 (1.08-8.52) based on eight exposed cases and eight exposed controls. A

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1 Recall bias occurs when cases are more likely to say they are exposed to glyphosate than controls or when controls are more likely to say they are exposed to glyphosate than cases. The recall must be different for the cases than the controls for this to cause a bias; errors in recalling past exposures that happen for both cases and controls would not be recall bias.

2 The odds ratio (OR) is calculated as the proportion of exposed cases with disease to exposed controls divided by the proportion of non-exposed cases to non-exposed controls. For rare diseases, this value approximates the population risk ratio (PRR) which is the probability of having the disease in exposed individuals divided by the probability of having the disease in non-exposed individuals. If the PRR is 1, then there is no difference in the probability of having the disease regardless of your exposure. Values of PRR greater than 1 imply the risk is higher in the exposed population. Because the OR is an estimate of the PRR for rare diseases, it is usually accompanied by a 95% confidence interval that describes the probable range of the estimate. If the OR is greater than 1, then the exposure is associated with the disease. If the lower 95% confidence bound for the OR is greater than 1, this is typically used to say the association is statistically significant.

more extensive analysis additionally controlled for other pesticides and yielded a smaller OR of 1.85 (0.55-6.20).

Eriksson et al. (2008)[15] conducted a population-based case-control study where cases were identified as NHL patients aged 18-74 years diagnosed in four major hospitals in Sweden from December 1, 1999 until April 30, 2002. The univariate analysis, adjusting for age, sex and year of diagnosis (cases) or enrollment (control) yielded an OR of 2.02 (1.10-3.71) based on 29 exposed cases and 18 exposed controls. When cases and controls were divided into those with ≤10 days per year exposure and those with >10 days per year exposure, the ORs were 1.69 (0.70-4.07) and 2.36 (1.04-5.37) respectively. They also did various subtypes of NHL and saw basically the same increased OR.

McDuffie et al. (2001)[16] recruited incidence cases of NHL in men 19 years or older from six Canadian provinces. The OR for glyphosate exposure and NHL was 1.26 (0.87-1.80) stratified by age group and province of residence and the OR was 1.20 (0.83-1.74) when the analysis also controlled for significant medical variables. The OR for exposure between zero to two days per year was 1.0 (0.63-1.57) and for greater than two days per year was 2.12 (1.20-3.73).

Orsi et al. (2009)[17] conducted a hospital-based case-control study of men and women diagnosed with lymphoid neoplasms in five hospitals in France. The OR for occupational use of glyphosate and NHL was 1.0 (0.5-2.2) with 12 exposed cases and 24 exposed controls stratified by age and center category. A further analysis was done by individual subtypes of NHL which yielded similar results.

Cocco et al. (2013)[18] evaluated data from a multi-center case-control study of lymphoid neoplasms in six European countries from 1998 to 2004. No results were provided for NHL and the only OR provided for glyphosate was for B-cell lymphoma where the OR was 3.1 (0.6-17.1) based on four exposed cases and two exposed controls. This study is of limited utility.

Pahwa et al. (2019) [19] pooled data from three case-control studies in the United States [8-10] and one case-control study from Canada [16] to form the North American Pooled Project (NAPP) data set. The crude OR for ever/never use of glyphosate was 1.43 (1.11-1.83 95% CI) and included 1690 cases and 5131 controls. This OR was attenuated in the pesticide-adjusted analysis (1.13, 0.84-1.51). Crude analyses for years of use showed increased ORs (<3.5 years, 1.59, 1.13-2.22; >3.5 years, 1.20, 0.82-1.75) with a p-trend of 0.05 and attenuated in the pesticide-adjusted analyses (<3.5 years, 1.28, 0.88-1.84; >3.5 years, 0.94, 0.62-1.42) with no apparent trend (p-trend=0.9). An ordinal analysis using 5-year increments was significant for the crude analysis (p=0.03) and attenuated in the pesticide-adjusted analysis (p=0.3). Days/year of use showed an increased OR in the highest exposure group in the crude analysis (≤2, 1.03, 0.67-1.60; >2, 2.42, 1.48-3.96) with a significant trend (p-trend=0.002) and again attenuated in the pesticide-adjusted analysis (≤2, 0.74, 0.46-1.19; >2, 1.73, 1.02-2.94; p-trend=0.2). An ordinal analysis with increments of 5/days per year was significant in the crude analysis (p=0.02) but not in the pesticide-adjusted analysis (p=0.2). Similar patterns were seen for total days handling glyphosate (crude; ≤3.5, 1.20, 0.74-1.95; >3.5, 1.55, 0.99-2.44, p-

trend=0.05, ordinal 10, p=0.02: pesticide-adjusted; ≤3.5, 0.87, 0.52-1.45; >3.5, 1.08, 0.66-1.77, p-trend=0.9, ordinal 10, p=0.08). Very similar patterns were seen for Diffuse large B-cell lymphoma (DLBCL) and small lymphocytic lymphoma (SLL) although the ORs for SLL seemed to be less attenuated when adjusted for the other 3 pesticides.

The limitations and strengths of all the case-control studies are discussed in Section 4.2.

III.b Cohort Studies

A cohort study follows a group of people with a common characteristic (e.g. farmers) for an extended period of time. At various points, the cohort is examined for various characteristics in the population (e.g. survival, smoking status, disease status) and exposures they may have encountered (e.g. pesticides). Statistical analyses can then be done to assess if an association exists between any exposure and any disease (e.g. between glyphosate use and NHL). Cohort studies generally do not suffer as much from recall bias but could have other deficiencies.

De Roos et al. (2005)[20] reported results on the association of glyphosate and cancer incidence from the Agricultural Health Study (AHS), a prospective cohort study in Iowa and North Carolina, which included 57,311 private and commercial applicators who were licensed to apply restricted-use pesticides at the time of enrollment. There were 92 cohort members with a diagnosis of NHL during the study period of which 77.2% had ever used glyphosate resulting in a rate ratio[3] (RR) of 1.2 (0.7-1.9) when controlling for age and an RR of 1.1 (0.7-1.9) when controlling for age, lifestyle factors, demographics and ten other pesticides. When cumulative exposure days in exposed individuals are divided into tertiles and RRs examined using the lowest exposed tertile as the reference group, the RRs drop with values of 0.7 (0.4-1.4) and 0.9 (0.5-1.6) for tertiles 2 and 3 respectively controlling for demographic and lifestyle factors and other pesticides (30,699 subjects). When intensity-weighted exposure days are examined again using exposed tertile 1 as the reference group, the RRs drop with values of 0.6 (0.3-1.1) and 0.8 (0.5-1.4) for tertiles 2 and 3 intensity-weighted exposure days respectively controlling for demographic and lifestyle factors and other pesticides (30,699 subjects).

Andreotti et al. (2017)[21] did a follow-up to the earlier report by **De Roos et al. (2005)**[20] which includes new cancers identified since the 2005 study and new information on exposure and usage. In addition to the original data on glyphosate use from **De Roos et al.** (**2005**), comprehensive use data was obtained by telephone questionnaire that was administered between 1999 and 2005. Only 63% of the cohort responded to the questionnaire so the authors used a multiple imputation procedure to impute glyphosate exposure for the remaining 37% of the cohort[22] prior to 2005. No rate ratio (RR) was provided for an ever-never use comparison. They evaluated cumulative exposure days in exposed individuals in quartiles and with all RRs below 1 but increasing with exposure with values of 0.73 (0.54-0.98), 0.80 (0.60-1.06), 0.86

[3] The rate ratio (RR) is estimated as the incidence in the exposed population divided by the incidence in the unexposed population. Incidence is calculated as the number of events in a fixed period of time divided by the person years at risk. Unlike the OR, the RR does not require the assumption of a rare disease to serve as a good estimate of the population risk ratio (PRR).

(0.65-1.15) and 0.78 (0.58-1.05) for quartiles 1,2,3 and 4 respectively controlling for numerous factors and other pesticides. Analyses were also presented for individual cancer classifications within the non-Hodgkin lymphoma family including B-cell NHL, chronic and small lymphocytic leukemia, diffuse large B-cell lymphoma, marginal-zone lymphoma, follicular lymphoma, multiple myeloma, and T-cell NHL. The results were similar for the subgroupings as they were for the combined NHL with the exception of T-cell NHL where the RRs for lifetime days of exposure were 3.83 (0.84-17.49) for exposure below the median with no lag, and 2.49 (0.95-6.57) for 20-year lag and for intensity-weighted lifetime days were 4.25 (0.73-24.64) for exposure below the median with no lag, and 2.97 (1.20-7.31) for 20-year lag based on a total of 22 cases. This study has a number of serious limitations as discussed in Section 4.3. These include exposure misclassification associated with the use of imputation for exposures in people who did not respond to questionaires and a rapidly changing pattern of use of glyphosate over the period when the questionnaire was being administered.

Leon *et al.* (2019) [23] formed the AGRICOH Consortium to evaluate the relationship of 33 pesticides (including glyphosate) with NHL in a pooled analysis of three large agricultural worker cohorts. The three cohorts were the AHS (described above for Andreotti et al. 2017), the AGRGICAN cohort [24] and the CNAP cohort [25]. While they used the AHS cohort, they excluded 4619 commercial applicators (non-farmers) used in the Andreotti et al. (2017) study and included 1620 farmers with no information on frequency of exposure that were excluded from Andreotti et al. (2017). The Agriculture and Cancer (AGRICAN) cohort consists of 181,747 farm owners and farm workers (male and female) over 18 years of age who were affiliated with the French national health insurance system for farm workers for at least 3 years and who resided in one of the 11 departments in France covered by population cancer registries. They were enrolled between 2005 and 2007 and cancer and mortality were assessed up to the end of 2009. The Cancer in the Norwegian Agriculture Population (CNAP) cohort consists of 147,134 farm-holders (owners and non-owners using a farm, male and female) who had participated in at least one of five national agricultural and horticultural censuses conducted in 1969, 1974, 1979, 1985 and 1989. The census included information on crops and livestock produced, acreage, technology, pesticide expenses and pesticide spraying equipment. Exposure to the various pesticides was assessed using crop-exposure matrices (CEM_2) specific to Norway that were derived based upon what chemicals were sold and registered for use in specific years. Cancers were assessed up to the end of 2011. AGRICAN had more females (44%) than the other two cohorts (16% CNAP and 3% AHS) while CNAP contributed the bulk of the person-years of follow-up with 2,396,595 person-years compared to 751,880 for AHS and 426,340 for AGRICAN. The largest crops reported differed also among the three cohorts with 70% of AGRICAN members reporting cultivating hay, meadows and grasslands, 32% in CNAP reporting potatoes and 74% of AHS reporting corn. The majority of the NHL cases were from CNAP (1498) with AHS (493) and AGRICAN (439) contributing many fewer cases. The fully-adjusted meta hazard ratio (mHR) for glyphosate and NHL was 0.95 (0.77-1.18) and the crudely-adjusted mHR was 0.98 (0.76-1.25). Separate analyses were also done for various subtypes of NHL. For chronic lymphocytic leukemia and small lymphocytic

lymphoma (CLL) the mHRs were 0.92 (0.69-1.24) fully adjusted and 1.09 (0.70-1.70) crude. For diffuse large B-cell lymphoma (DLBCL) the mHRs were 1.36 (1.00-1.85) fully adjusted and 1.12 (0.86-1.45) crude. For follicular lymphoma (FL) the mHRs were 0.79 (0.52-1.21) fully adjusted and 0.95 (0.70-1.45) crude. For multiple myeloma and plasma-cell leukemia (MM) the mHRs were 0.87 (0.66-1.15) fully adjusted and 1.00 (0.83-1.21) crude. There was evidence of heterogeneity among the three cohorts for NHL (I^2=57%) but no heterogeneity among the cohorts for the various subtypes of NHL.

Limitations and strengths of all the cohort studies are described in detail in Section 4.3.

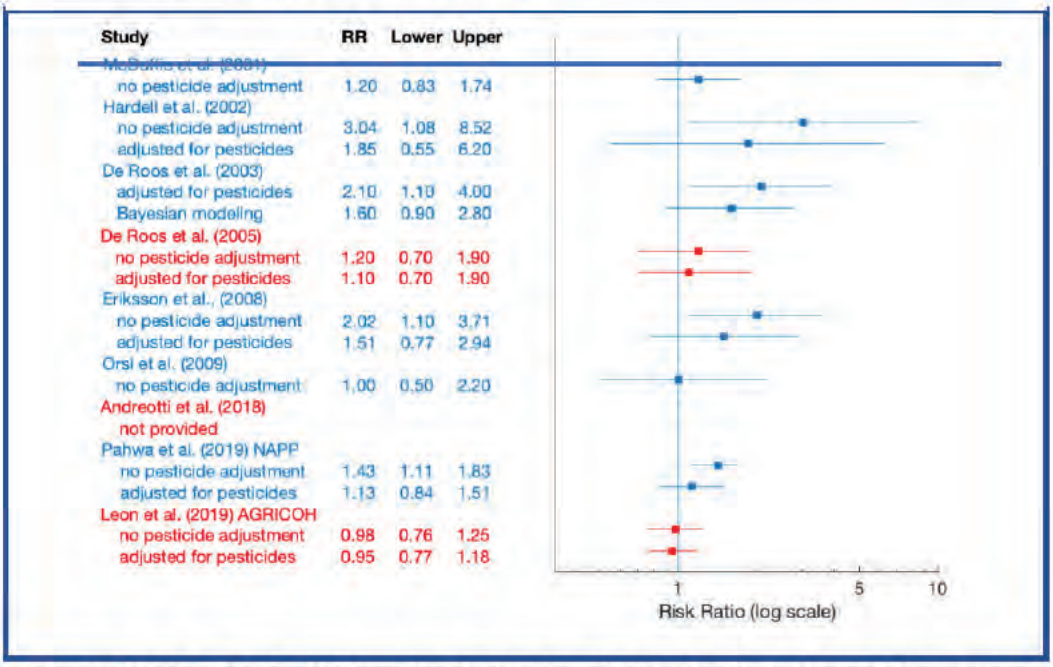
IV. Consistency of Associations in Human Epidemiology Data

There is a consistency of associations across the better epidemiology studies.

Hill (1965)[4] defines consistency as the answer "yes" to the question "Has it repeatedly been observed by different persons, in different places, circumstances and times?" For these studies, the answer is indeed yes. Consistency of the associations across several epidemiology studies is not simply a matter of seeing how many were statistically significant and how many were not but must also address the consistency of the direction of the responses.

Figure ES- 1 shows a forest plot of all ORs and RRs for ever/never exposure from the epidemiology studies discussed previously. The nine studies shown in Figure ES-1 will be referred to as the nine core epidemiology studies. Eight of these studies provide an OR/RR number for ever/never exposure to glyphosate. Each horizontal line in the forest plot shows the mean estimate of the OR/RR as a black square and the 95% confidence interval around this estimate as whiskers extending left and right from the black square. The first obvious conclusion to be drawn from Figure ES-1 is that all of the mean OR/RR estimates (solid squares) for the case-control studies (blue text and lines) and the **DeRoos et al (2015)** [20] cohort study are consistently ≥1; the only exception is **Leon et al. (2019)** [23]. The probability of this happening if the underlying PRR=1 (no association) is 0.035, suggesting they consistently support a positive effect.

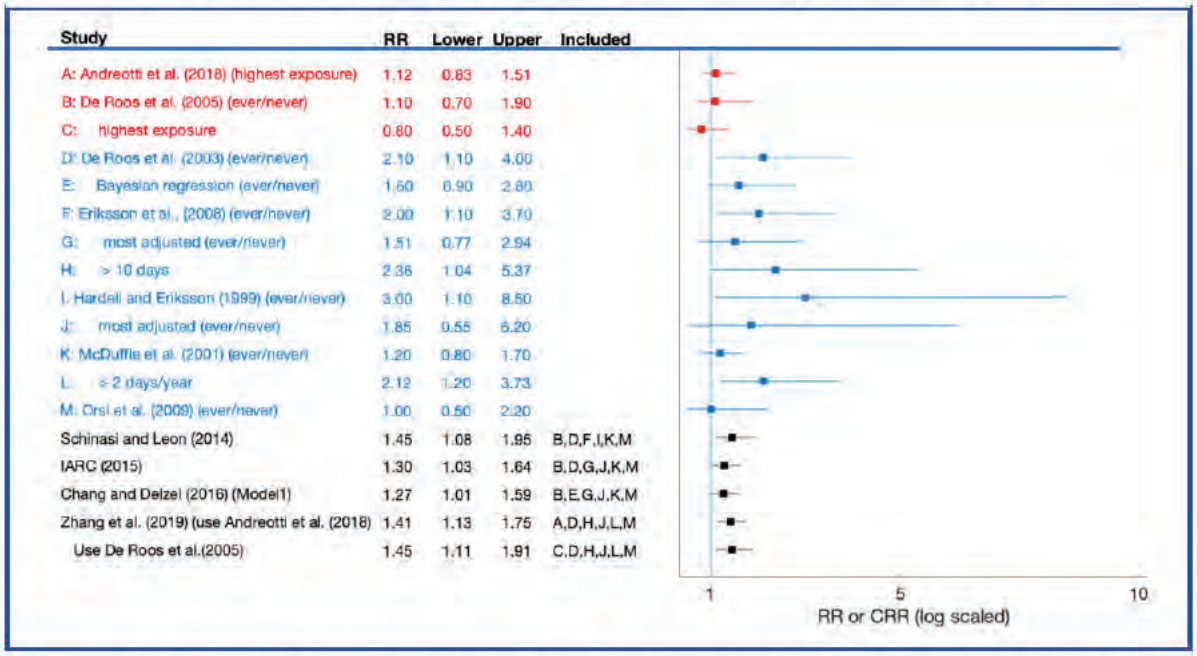
Figure ES- 1: Odds Ratios and Rate Ratios for ever/never exposure from selected epidemiology studies.



("RR" refers to the OR, RR or mHR from the study, "Lower" refers to the 95% confidence lower bound and "Upper" to the 95% confidence upper bound. Case-control studies are shown in blue and cohort studies in red.)

Consistency can be evaluated by combining the individual studies using meta-analysis to obtain a combined analysis using both the ORs and the RRs (CRR) and test for heterogeneity in the studies. Four meta-analyses have been conducted for different combinations of the studies shown in Figure ES-1; **Chang and Delzell (2016)** [7], **IARC (2015)** [5], **Schinasi and Leon (2014)** [26], and **Zhang et al. (2019)** [27]. **Chang and Delzell (2016)** did four separate meta-analyses on the glyphosate epidemiology studies using two different methods (random-effects and fixed-effects models); their model number 2 matched exactly what was done by **IARC (2015)**. Figure ES- 2 shows a forest plot with the principle analyses from each of these meta-analyses and the underlying individual evaluations from the individual studies. The analyses by **IARC (2015)** [5] and **Schinasi and Leon (2014)** [26] are effectively covered by the analyses of **Chang and Delzell (2016),** so I will focus on only **Chang and Delzell (2016)** [7] and **Zhang et al. (2019)** [27].

Figure ES- 2: Odds Ratios and Rate Ratios for ever/never exposure from selected epidemiology studies and the meta-analyses done on these studies.



("RR" refers to the OR or RR from the study, "Lower" refers to the 95% confidence lower bound and "Upper" to the 95% confidence upper bound. Case-control studies are shown in blue, cohort studies in red and meta-analyses in black.)

Chang and Delzell (2016), combined the studies in several different ways, all of which lead to confidence bounds on the combined risk ratios (CRR) excluding 1.0 and being greater than 1.0. They tested for heterogeneity with each model and saw no heterogeneity across the studies. Thus, every meta analysis they performed showed a statistically significant association between use of a glyphosate formulation and NHL.

Chang and Delzell (2016) also evaluated the association between subtypes of NHL and glyphosate exposure where possible. They saw significant positive associations for B-cell lymphomas and non-significant increases for all other sub-types. **Chang and Delzell (2016)** also performed a sensitivity analysis by only doing meta-analyses on studies with similar characteristics. Using only the five case-control studies, the CRR was 1.3 (1.0-1.7). Breaking them into the type of control used, there were four studies using population controls with a CRR of 1.4 (1.0-1.8). There were four studies with males only with a CRR of 1.3 (1.0-1.7) and two studies with males and females with a CRR of 1.2 (0.8-1.8). Three studies were done in North America with a CRR of 1.2 (1.0-1.6), three in Europe with a CRR of 1.3 (0.8-2.1); two of the three studies were in Sweden with a CRR of 1.6 (0.9-2.8).

Zhang et al. (2019) [27] conducted a meta-analysis that includes the study by **Andreotti et al** (2018) [21] using the highest exposure groups, when available, from each study (seeFigure ES- 2). They saw an increased CRR of 1.41 (1.13-1.75) for the fixed effects model and 1.52 (1.12-2.16) for the random effects model. When they used the highest exposures from the **DeRoos et al (2005)** study rather than **Andreotti et al. (2018)**, the CRRs were 1.45 (1.11-1.91) for the fixed effects model and 1.52 (1.00-2.31) for the random effects model. Using the longest duration rather than the highest exposure

yielded similar results. Excluding the AHS study and only including the highest exposures from the case-control studies yielded CRRs of 1.84 (1.33-2.55) for the fixed effects model and 1.86 (1.39-2.48) for the random effects model. They also did a variety of sensitivity analyses to determine if the results were sensitive to the inclusion of certain studies, different exposure measures, different geographical regions, etc. In all cases, the resulting CRR was greater than 1 and in nearly all cases had a 95% lower confidence bound above 1.

No published meta-analysis are available that include the **Leon et al. (2019)** and **Pahwa et al. (2019)** studies. Because the **Pahwa et al (2019)** study is positive for a linkage between NHL and glyphosate exposure, it is unlikely to substantively change the meta-analyses seen above. It is unclear what effect the **Leon et al. (2019)** study would have on a meta-analysis against the other studies. However, given its unique use of CEMs to estimate exposures and the problems associated with the **Andreotti et al. (2018)** study discussed in Section 4.3, a meta-analysis using these data should carry less weight in the overall evaluation.

In case-control studies, it is possible that in a few of these studies, the method by which controls were selected could contribute to selection bias that might lead to increased ORs. However, given the diverse types of cases and controls used in the five core case-control studies, this is unlikely to explain the consistent findings seen from these studies.

Exposure misclassification can lead to increases or decreases in the OR or RR values seen in both case-control and cohort studies. In all of the core epidemiological studies, the authors concluded there was bound to be some exposure misclassification, but that it was most likely non-differential, meaning that the misclassification was random; this would likely reduce the OR/RRs seen in the studies rather than increase them. However, for the **Andreotti et al (2018)** study, it is possible there is some differential exposure misclassification as discussed in Section 4.3.

Confounding occurs when there is an exposure or some other factor that is tightly associated with both glyphosate exposure and NHL diagnosis that, if controlled for, could explain the results. The most likely source of confounding in these studies would be exposures to other pesticides. Seven [11, 14, 15, 19-21, 23] of the core studies controlled for exposure to other pesticides.

In conclusion, we have seven epidemiology studies done on two different continents by different research groups using different designs, questionnaires and study populations that are very consistent with no obvious bias or confounding that would explain the results. There are two additional studies, Andreotti et al. (2018) and Leon et al. (2019) that are not independent, have problems with exposure misclassification and differ from the other seven in response. **There is a consistency of associations across the better epidemiology studies.**

V. Strength of Associations seen in Human Epidemiological Studies

There is a strong association across the better epidemiology studies.

To explain strength of association, **Hill (1965)** gives the classic example of John Snow and the cholera epidemic of 1855 where the risk ratio of dying if you drank water from the Southwark and Vauxhall Company (polluted by sewage) compared to drinking from the Lambeth Company water (sewage free) was 14. Yet, for the six core studies, the OR/RR for ever/never exposure ranges from 1.0 to 1.85 for the most-fully-adjusted risk estimates and to 2.1 if you include the fully adjusted risk estimate from De Roos et al. (2003)[20] using logistic regression. These are moderate OR/RR estimates making it conceivable they are individually due to either chance or bias. Thus, with the exception of the logistic regression analysis in **De Roos et al. (2003)** [20], none of the core studies demonstrate large, precise risks as envisioned by **Hill (2016) [4]**. However, **Hill (1965)** was not expressing himself in statistical terms where the significance of an association is dependent upon the precision of the observations. The meta-analyses shown in Figure ES- 2 all demonstrate estimates of CRR that are significantly different from 1 rejecting the concept that the overall association is due to chance. The statistically significant estimates of the CRR for B-cell lymphomas in the meta-analyses also support this finding as does the NAPP pooled analyses demonstrating consistent positive findings.

In summary, we have seven of the nine core epidemiology studies that all show approximately the same, modest increase in OR/RR that, when combined, demonstrate a significant strength of association. **There is a strong association across the better epidemiology studies.**

VI. Biological Plausibility

The evidence for biological plausibility is overwhelming. Glyphosate clearly causes multiple cancers in mice, two cancers in the hematopoietic system similar to what is seen in humans, causes cancer in rats, is genotoxic and induces oxidative stress. The findings are clear for both glyphosate alone and for glyphosate formulations. There is strong support for biological plausibility in support of a causal association of glyphosate and glyphosate formulations with NHL.

The range of data one can use to determine biological plausibility is quite diverse and can be exceptionally complicated. For simplicity, it can be divided into the types of assays that can be used in this evaluation: animal cancer bioassays, toxicokinetic studies, studies from accidental exposures in humans, and studies of specific biological mechanisms in animals or cells derived from humans or animals.

VI.a Animal Cancer Bioassays

VI.a.1 General Introduction

Typical animal cancer bioassays will expose animals (generally rats or mice) to a chemical for a substantial proportion of the animal's life (generally 2 years, sometimes 18 months) then kill the animal and examine its organs and tissues for tumors. There are guidelines on how to conduct and analyze these studies. Typically, chemical registrants conduct cancer bioassays for pesticide approval pursuant to guidelines developed under

the guidance of the Organization for Economic Cooperation and Development (OECD[28]).

Studies generally use four groups of animals, one group receiving no exposure (control) and the remaining three groups are test animals, with each group receiving different dose exposures to the chemical[29]. Doses generally above human experience are used in animal carcinogenicity studies because only relatively small numbers of animals are being used to evaluate risk for a large human population and because even the best known human carcinogens do not cause cancer in large fractions (say 20%) of the human population. The basic underlying premise of this design consideration is that, as the dose increases, so does the risk of getting a tumor. By exposing animals to the highest dose possible, you increase the ability of the study to identify a risk if one is present. However, one must be careful not to use a dose that is so high it will cause cancers by processes that would never work at lower doses. To avoid this, studies are designed around a maximum tolerated dose (MTD). This dose is generally determined based upon a subchronic study (90 days) in the same animals and is usually the maximum dose that can be tolerated by the animals without any signs of significant toxicity in the exposed animals (e.g., weight loss, tissue damage). The OECD and EPA provide guidelines[3, 28] on how to choose this top dose.

There are 14 animal carcinogenicity studies in rats[30-43] and nine in mice [44-52]. Only two studies[33, 39] appear in the peer-reviewed literature; the remaining studies are partially available through several sources. For seven of the rat studies [30, 31, 34, 36, 40-42] and five mouse studies [44, 46, 47, 50, 51], confidential proprietary technical reports from the performing laboratory are available following the findings of court case T-329/17 in Europe[4] [53]. For the remaining studies, data was obtained from the EPA review of glyphosate [54], the European Food Safety Authority review of glyphosate [55, 56], the Joint Meeting on Pesticide Residues report on glyphosate [57] and supplemental material from a review of the carcinogenicity of glyphosate by a panel of scientists on behalf of Monsanto[58].

Detailed methods for the analyses of the individual studies are provided in Section 7.1.1. The Cochran-Armitage trend test [59] is used for individual tumor counts in individual studies. Logistic regression is used to evaluate the consistency of a tumor finding across multiple studies using the same sex-species-strain combinations. In some cases, tumors that rarely (<1% in untreated animals) appear in laboratory animals can be increased but do not show statistical significance. Most guidelines call for the use of historical control data to evaluate these cases to assess the significance of the findings [2, 3, 28]. For these evaluations, the test proposed by Tarone [60] is used with an appropriate historical control group as discussed in the text.

To summarize the strength-of-evidence for each tumor, four categories are used. Clear evidence (CE) is indicated when the data demonstrate a causal linkage between glyphosate and the tumor based upon the reanalysis in this review and the available peer-reviewed literature. Some evidence (SE) is indicated when the data demonstrate a

[4] See http://curia.europa.eu/juris/documents.jsf?num=T-329/17

linkage between glyphosate and the tumor but chance, although unlikely, cannot be ruled out. Equivocal evidence (EE) also indicates the data demonstrate a linkage between glyphosate and the tumor but chance is as likely an explanation for the association as is glyphosate. No evidence (NE) indicates any linkage between glyphosate and the tumor based upon the reanalysis in this review is almost certainly due to chance. The factors used to put tumors into these categories include the analyses of the individual studies, the consistency of the data across studies (the pooled analyses), the analyses using historical control data, the analyses of the non-neoplastic lesions, the mechanistic evidence and the associated scientific literature.

VI.a.2 Evaluation of Carcinogenicity Studies

There are a total of 41 positive tumor findings ($p<0.05$) in the 13 animal carcinogenicity studies evaluated for this report. Many of these (19) are highly significant ($p<0.01$). Some of the same tumor types are seen in multiple studies, in two strains of the same species, and in two different species. Several tumors are rare tumors (occur spontaneously less than 1% of the time) and their significance was validated against historical data.

Increases in kidney adenomas and carcinomas (combined) are seen in male CD-1 mice and increases in adenomas are seen in Swiss albino mice and male SD rats. Dose-related increases in malignant lymphomas are seen in male and female CD-1 mice and marginal increases are seen in male and female Swiss albino mice. Increases in hemangiosarcomas are seen in male CD-1 mice. Skin keratoacanthomas are increased by glyphosate in male SD rats and male Wistar rats. Skin basal-cell tumors are also increased in male SD rats. Hepatocellular adenomas are increased by exposure to glyphosate in male SD rats and Wistar rats. Adrenal cortical carcinomas are increased in female Sprague-Dawley rats. There is also a suggestion of an increase in adrenal pheochromocytomas in male Wistar rats and of pituitary adenomas in male and female Wistar rats. There is an inconsistent effect of glyphosate on the rates of mammary gland adenomas, carcinomas and combined adenomas and carcinomas in female Wistar rats but not in SD rats. There is an inconsistent increase in thyroid C-cell adenomas and/or carcinomas in male and female SD rats and thyroid follicular cell adenomas in male SD rats. An inconsistent finding of an increase in lung tumors was seen in male and female CD-1 mice and Harderian gland tumors in female CD-1 mice. An increase in hemangiomas was seen in female CD-1 and Swiss-Albino mice. Pancrease islet-cell tumors were increased in male SD rats. Finally, testis interstitial-cell tumors were increased in one study in male SD rats.

Many of these tumor findings were supported by pre-neoplastic lesions and organ toxicity in the same tissues in these studies. There is also a substantial amount of data in the peer-reviewed literature that supports these findings. An analysis of the probability that all of these tumors arose by chance (Section 7.1.6) clearly indicates this is highly unlikely.

Considering the analyses of the individual studies, the consistency of the data across studies (the pooled analyses), the analyses using historical control data, the analyses of

the non-neoplastic lesions, the mechanistic evidence and the associated scientific literature, a strength-of-evidence can be assigned to each tumor finding (summarized in Table 19).

There is clear evidence that glyphosate causes hemangiosarcomas, kidney tumors and malignant lymphomas in male CD-1 mice and hemangiomas and malignant lymphomas in female CD-1 mice. There is clear evidence that glyphosate causes hemangiomas in female Swiss albino mice. There is clear evidence that glyphosate causes kidney adenomas, liver adenomas, skin keratoacanthomas and skin basal cell tumors in male Sprague-Dawley rats and adrenal cortical carcinomas in female Sprague-Dawley rats. There is clear evidence that glyphosate causes hepatocellular adenomas and skin keratocanthomas in male Wistar rats.

There is some evidence that glyphosate causes malignant lymphomas in male and female and kidney tumors in male Swiss albino mice. There is some evidence that glyphosate causes testicular interstitial cell tumors in male Sprague-Dawley rats. There is some evidence that glyphosate causes pituitary adenomas in male and female Wistar rats and mammary gland adenomas and carcinomas in female Wistar rats.

There is equivocal evidence that glyphosate causes thyroid c-cell adenomas and carcinomas in male and female Sprague-Dawley rats, and thyroid follicular cell adenomas and carcinomas and pancreas islet-cell adenomas in male Sprague-Dawley rats. There is equivocal evidence glyphosate causes adrenal pheochromocytomas in male Wistar rats.

There is no evidence that glyphosate causes lung tumors in male and female CD-1 mice or Harderian gland tumors in female CD-1 mice.

Glyphosate causes cancer in male and female, rats and mice. Two cancers seen in the hematopoietic systems of male mice are similar to what is seen in humans

Table ES- 1: Summary of level of evidence1 for tumors observed to have a significant trend in 13 rodent carcinogenicity studies in male and female, mice and rats.

Tumor	Males				Females			
	SD Rat	Wistar Rat	CD-1 Mouse	Swiss Mouse	SD Rat	Wistar Rat	CD-1 Mouse	Swiss albino mouse
Adrenal cortical carcinoma					CE			
Alviolar-Bronchiolar tumor			NE				NE	
Harderian gland tumor							NE	
Hemangioma							CE	CE
Hemangiosarcomas			CE					
Kidney tumor	CE		CE	SE				EE
Liver Adenoma	SE	CE						
Mammary tumor						SE		
Malignant lymphoma			CE	SE			CE	
Pancreas Islet Cell Tumor	SE							
Pituitary tumor		SE				SE		
Skin basal-cell tumor	CE							
Skin keratoacanthoma	CE	CE						
Thyroid C-cell tumor	EE				EE			
Thyroid follicular-cell tumor	EE							
Testis interstitial-cell Tumor	SE							

1 – criteria as defined in Section 7.1.1: CE=clear evidence; SE=some evidence; EE=equivocal evidence; NE=no evidence

VI.b Mechanisms Relating to Cancer

VI.b.1 Genotoxicity

Genotoxicity refers to the ability of an agent (chemical or otherwise) to damage the genetic material within a cell, thus increasing the risks for a mutation. Genotoxic substances interact with the genetic material, including DNA sequence and structure, to damage cells. DNA damage can occur in several different ways, including single- and double-strand breaks, cross-links between DNA bases and proteins, formation of micronuclei and chemical additions to the DNA. Just because a chemical can damage DNA does not mean it will cause mutations. So, while all chemicals that cause mutations are genotoxic, all genotoxic chemicals are not necessarily mutagens.

This is a complicated area from which to draw a conclusion due to the diversity of the studies available (there are multiple species, multiple strains within a species, multiple cell types from multiple species, differing lengths of exposure, differing times of evaluation after exposure, differing exposures, numerous markers of genotoxicity, and finally both glyphosate and multiple different glyphosate formulations). There are three studies that evaluate the genotoxicity of glyphosate in humans directly, 36 experiments in eight strains of mice, four studies in rats, 14 studies in human lymphocytes and 18 studies in other human cells, 12 studies in non-human mammalian cell lines (two using mouse cells, five using hamster cells, two using rat cells and three using cells from cows), a large number of studies in a wide variety of non-mammalian species, and a plethora of studies, mostly identical, in bacteria.

Some conclusions are straightforward"; glyphosate does not appear to cause reverse mutations for histidine synthesis in *Salmonella typhimurium*, regardless of whether these reverse mutations are due to frameshift mutations or point mutations. There is ample evidence supporting the conclusion that glyphosate formulations and glyphosate can cause genotoxicity in non-mammalian animal species. This clearly indicates that both glyphosate and the formulations are able to cause injury to DNA.

The more important studies are those that have been done using mammalian systems, human cells and direct human contact. Table ES- 2 summarizes these studies in a simple framework that allows all of the experimental data to be seen in one glance. This table does not address the subtlety needed to interpret any one study, but simply demonstrates when a study produced positive versus negative results.

Clearly, for *in vitro* evaluations in human cells, the majority of the studies have produced positive results. The majority of these studies used either the comet assay (a simple way for measuring any type of DNA strand break) or methods that counted specific types of strand breaks in the cells (e.g. SCEs, micronuclei, nuclear buds and nucleoplasmic bridges). From these assays, we can conclude there is DNA damage. For glyphosate formulations, there are only three studies in humans *in vivo*, two of which were positive. In human cell lines, there are 5 positive studies and no negative studies using GBFs.

The magnitude of the concentrations used in these studies could potentially lead to false positives if the glyphosate is causing cytotoxicity in the cells. All studies using the comet assay were positive with no study showing a negative response above 10 µg/ml and mixed results below that with positive results at 0.12 and 3.5 µg/ml and negative results at 2.91 and 10 µg/ml. In general, the comet assays provide strong support for genotoxicity.

The studies that directly addressed specific types of strand breaks in cells following exposure to glyphosate showed markedly different responses across the various concentrations used. While changes have been seen in six of the seven studies, the actual concentrations in which the changes are seen is not consistent across studies. Even with these inconsistencies, I conclude that glyphosate causes DNA strand breaks, which is indicative of genotoxicity.

Table ES- 2: Summary of in vivo and in vitro genotoxicity studies of glyphosate and glyphosate formulations in mammals[1]

In vivo or *in vitro*	Species	Cell type or tissue	Glyphosate[2]		Glyphosate Formulations	
			Number Positive	Number Negative	Number Positive	Number Negative
In vivo	Humans	Peripheral blood			2	1
in vitro	Humans	lymphocytes	8	2(1)	4	
		semen		1		
		cell lines	8	1	6	2
In vivo	Swiss CD-1 Mouse	Liver/Kidney	1	1	2	
	Wistar Rats	Liver/blood	1			
In vivo (micro-nucleus assay)	NMRI mouse	Erythrocytes		4(3)		2(1)
	Swiss CD-1 mouse		1		2	
	Balb C mouse		1			
	$B6C3F_1$ mouse			1		
	Swiss mouse		1(1)			3(2)
	CD-1 mouse		2(2)	1(1)	2 (2)	6 (6)
	Swiss albino mouse		1(1)	3(3)	1	
	C57BL mouse					1
	Mouse (not specified)				1	
	Rats (all)			2(1)		1(1)
In vitro	Mouse	L5178 lymphoma		2(2)		
	Chinese hamster	Lung		3(3)		
	Chinese hamster	ovary	1	1		
	Fischer rat	liver		1		
	Rat	Lymphocytes		1(1)		
	Bovine	Lymphocytes	1		2	

[1]each entry in the table corresponds to a single study where a study is positive if at least one valid positive finding emerged from the study $p<0.05$; entries in the table are only for studies where data was available to review including data from EFSA[55] and Kier and Kirkland (2000)[61]; [2]numbers are the total number of studies in this category, numbers in parentheses are the subset of studies that are regulatory studies

The studies that directly addressed specific types of strand breaks in cells following

exposure to glyphosate showed markedly different responses across the various concentrations used. While changes have been seen in six of the seven studies, the actual concentrations in which the changes are seen is not consistent across studies. Even with these inconsistencies, I conclude that glyphosate causes DNA strand breaks, which is indicative of genotoxicity.

The micronucleus assays in rodents examining glyphosate genotoxicity are either all positive in one strain or all negative in one strain with the exception of the three studies in CD-1 mice and four studies in Swiss Albino mice. For the positive studies, we can ask the question of whether, in this strain, the actual number of micronuclei are consistent.

In Swiss Albino mice, all four studies were done with males and females. Exposures were by oral gavage for the positive study (in female mice) and IP injection by the negative studies. The positive study was at 5000 mg/kg and the highest dose in any of the negative studies was 3024 mg/kg. Finally, the control response in the positive study was 6.7 micronucleated PCE per 1000 PCE whereas the controls in the three negative studies were between 0 and 0.6 micronucleated PCE per 1000 PCE. Any of these differences could easily explain the differences in response so the positive result in Swiss Albino mice should be accepted.

For CD-1 mice, the one negative micronucleus study was by oral gavage in males and females at a single dose of 5000 mg/kg. One of the positive studies was also by oral gavage in males at a single dose of 2000 mg/kg. Because of the nature of statistical noise, these two studies could both occur whether there is a true effect or not. For the other positive study, the dose was by IP injection in male mice with a positive response at 600 mg/kg that was more than double the response of the controls. These data support the finding that glyphosate can cause micronuclei in male CD-1 mice, which is indicative of genotoxicity.

The remaining *in vitro* assays in mammalian cells exposed to glyphosate show mixed results. In general, these responses appear to be negative with the exception of those seen in bovine lymphocytes that appear to show a positive increase in SCEs following exposure to glyphosate.

For glyphosate formulations, the main difference between the findings for glyphosate and those for the glyphosate formulations is the direct evidence for genotoxicity in humans and the micronucleus assays in Swiss mice. The observation of genotoxicity in humans following exposure to glyphosate formulations must carry the greatest weight in the overall analysis and two of the three studies were positive with the strongest study by **Bolognesi et al. (2009)**[62] showing the strongest response.

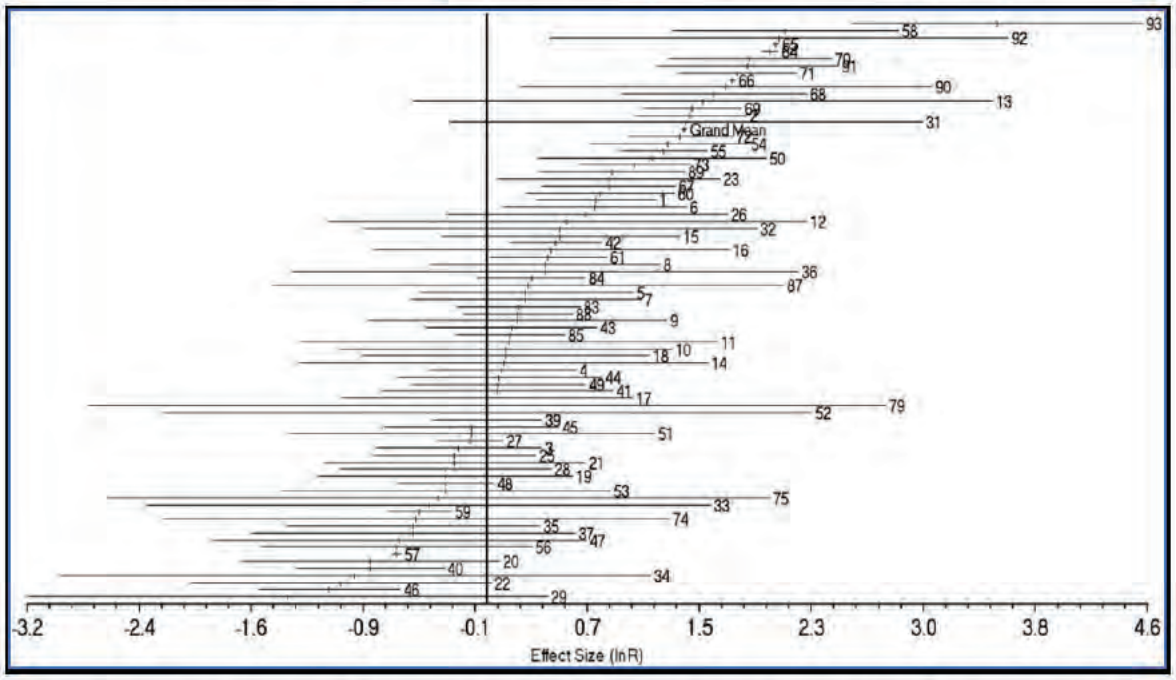
For the Swiss mouse studies of micronuclei, the fact that all three studies are negative for glyphosate formulations while one study is positive for glyphosate creates a clear disagreement. The positive study is an oral gavage study with an effect seen in male mice at 30 mg/kg/day. The two negative regulatory studies for glyphosate formulations were done at 2000 mg/kg (about 500 mg/kg glyphosate equivalent), were also oral gavage studies and were replicates done in the same laboratory at different times. The remaining negative study used glyphosate formulation doses of 50-200 mg/kg (25-100

mg/kg glyphosate equivalent) but was done by intraperitoneal injection. With the exception of the different routes of exposure, the differences between these studies cannot be resolved.

Ghisi et al. (2016)[63] did a systematic search to identify all published studies evaluating the ability of glyphosate or glyphosate formulations to induce micronuclei *in vivo*. An experiment, in their evaluation, was defined by sex/species/route/form of glyphosate so that some studies doing both sexes using glyphosate and a glyphosate formulation will enter multiple times into the analysis. They identified 93 experiments from which it was possible to do a meta-analysis. Data were extracted for each study and the log ratio of the mean of each experimental group to the mean control response (E+) was used to evaluate effect sizes in the meta-analysis. For this meta-analytic mean, a value below zero suggests no genotoxicity while a value above zero suggests increased genotoxicity. A test of heterogeneity (Cochran's Q statistic discussed earlier for the epidemiological data) was also evaluated.

Figure ES- 3 is a reprint of Figure 1 from the study by **Ghisi et al. (2016)**[63] and is a forest plot from all studies they evaluated for glyphosate and glyphosate formulations. It is clear from this plot that the predominant response is positive in these data with an overall grand mean response across all studies of E+=1.37 and a 95% confidence interval of (1.356-1.381) (this is highly statistically significant with a p<0.0001). The Qt value for the grand mean was also statistically significant suggesting there are other explanatory variables in the data that would help to explain the overall variance.

Figure ES- 3: Forest plot of studies evaluating micronucleus frequency in glyphosate exposure, arranged by effects size [Reprinted from Ghisi et al. (2016)].



The plot shows the estimate of the response ratio and 95% confidence interval (CI) of each experiment included in the meta-analysis. The number beside the bars represents the reference number of each experiment as in Table 1 of Ghisi et al. (2016)[63]. Grand Mean is the overall mean effects size of all

studies. [Reprinted from Ghisi et al. (2016)[63]]

Categorical variables were then used to make comparisons across the various strata in the data to identify which experimental conditions show the largest impacts on the mean response. Mammalian species presented a higher mean effect (E+=1.379; 1.366-1.391) than non-mammalian species (E+=0.740; 0.641-0.840). Glyphosate formulations showed a greater mean response (E+=1.388; 1.375-1.400) than did glyphosate (E+=0.121; 0.021-0.221), but both were significantly greater than zero. The mean response in studies using only male animals (E+=1.833; 1.819-1.847) was significantly different from zero as were studies using both males and females (E+=0.674;0.523-0.825) whereas the mean response in studies using only females (E+0.088; -0.153-0.328) was not. Peer-reviewed studies had higher mean response (E+=1.394; 1.381-1.407) compared to regulatory studies (E+=0.114; 0.027-0.202), but both means were significantly greater than zero, indicating an overall genotoxic effect. Other variables were examined such as length of exposure and magnitude of exposure that had very little impact on the overall findings.

In summary, the data support a conclusion that both glyphosate and glyphosate formulations are genotoxic. Thus, there is a reasonable mechanism supporting the increases in tumors caused by glyphosate and glyphosate formulations in humans and animals.

VI.b.2 Oxidative Stress

Oxidative stress refers to an imbalance between the production of reactive oxygen species (free radicals) in a cell and the antioxidant defenses the cell has in place to prevent this. Oxidative stress has been linked to both the causes and consequences of several diseases[64-69] including cancer[70-75]. Multiple biomarkers exist for oxidative stress; the most common being increased antioxidant enzyme activity, depletion of glutathione or increases in lipid peroxidation. In addition, many studies evaluating oxidative stress used antioxidants following exposure to glyphosate to demonstrate that the effect of the oxidative stress can be diminished.

Eight studies addressed oxidative stress in human cells and another ten studies addressed it in mammalian systems. In lymphocytes and erythrocytes from healthy donors, oxidative stress was detected as low as 580 µg/ml in lymphocytes and at 42.3 µg/ml in erythrocytes. In HepG2 cells, no increased oxidative stress was seen for a single concentration of 900 µg/mL, however, in a second study, some markers of oxidative stress were significantly changed at doses as low as 2.91 µg/mL. In two studies in HaCat cells, glyphosate induced oxidative stress in a continuous model fit to the results in one study and at the lowest concentration (1700 µg/ml) in the other. The most convincing studies in human cells for oxidative stress are the two studies in human blood.

In Swiss CD-1 male mice, increased oxidative stress was seen in the liver at 24 hours, but not at four hours after injection of 300 mg/kg glyphosate. No increase was seen in the kidney. In Wistar rats, repeated IP dosing with glyphosate lead to increased oxidative

stress in multiple organs using multiple biomarkers. Thus, all of the laboratory studies demonstrated oxidative stress with a significant finding in the rat study.

In Hep-G2 cells, a glyphosate formulation demonstrated a robust increase in oxidative stress at 40 µg/ml. Given the negative response in this cell line for glyphosate alone, it must be concluded that this response is not due to glyphosate. In HEP-2 cells, a glyphosate formulation demonstrated a robust increase in oxidative stress via multiple biomarkers at 376 µg/ml and when a surfactant is added, at 180.2 µg/ml. In HaCaT cells, a glyphosate formulation demonstrated significant increases in oxidative stress from doses starting as low as 1.7 µg/ml in a concentration-dependent fashion. No studies were available in human lymphocytes.

In Swiss CD-1 mice, a glyphosate formulation significantly increased oxidative stress in the kidney but only demonstrated a mild (non-significant) increase in the liver. This study evaluated oxidative stress at two different time points following exposure and saw responses that differed over time. The strong increase in the liver for glyphosate but not glyphosate formulation, suggests a complicated response pattern for pure glyphosate versus the formulation that could be linked to the time since exposure. In Swiss Albino mice, a glyphosate formulation demonstrated increased oxidative stress by two separate biomarkers in both the liver and the kidney. In a second study in Swiss albino mice using a different biomarker but a similar dose, increased oxidative stress was seen in both the liver and the kidney. In Wistar rat pups exposed in utero, an increase in oxidative stress was seen in the hippocampus and, in second study, in brain. In male Wistar rats, oxidative stress was clearly increased in the testis. In Swiss albino mice, topical application of a glyphosate formulation to the skin resulted in a proteomic fingerprint suggesting oxidative stress was increased. In Sprague-Dawley rats, glyphosate increased oxidative stress in the kidney, liver and blood.

Though there are fewer studies for oxidative stress than there are for genotoxicity, the robust response seen here in human cells and in rodent studies clearly supports a role for both glyphosate and glyphosate formulations in inducing oxidative stress. Thus, there is a second reasonable mechanism through which the tumors seen in humans and those seen in animals can be caused by glyphosate and glyphosate formulations.

VI.c Summary for Biological Plausibility

In the evaluation of causality, the evidence for biological plausibility is overwhelming. Glyphosate clearly causes multiple cancers in mice, two cancers in the hematopoietic system similar to what is seen in humans, causes cancer in rats, is genotoxic and induces oxidative stress. The findings are clear for both glyphosate alone and for glyphosate formulations. **There is strong support for biological plausibility in support of a causal association of glyphosate and glyphosate formulations with NHL.**

VII. Biological Gradient

A biological gradient exists for both the epidemiological data and the animal

carcinogenicity data, thus there is support for a biological gradient.

Five of the epidemiological studies provided information on biological gradients in their publications.

Eriksson et al. (2008)[15] and **McDuffie et al. (2001)**[16] had consistent results for intensity of exposure per year (e.g. >2 days per year is highly significant). **Pahwa et al. (2019)** [19] combined these studies and saw an increase for less that 3.5 years of exposure (OR 1.59 unadj., 1.28 adj.) and this dropped for >3.5 years of exposure (1.20 unadj., 0.94 adj.). Breaking exposure at 2 days per year there was clear dose-response (≤2, OR 1.03 unadj', 0.74 adj.; >2 days 2.42 unadj., 1.73 adj.). Both studies from the AHS [20, 21] showed no dose-response for any of their exposure metrics. However, the high frequency of exposure to many pesticides means subjects with low exposure to glyphosate were likely to be exposed to other agents that replace glyphosate and may also induce NHL; this could reduce the RRs in the higher exposure classes because it would inflate the RR in the low-exposure referent group.

It is not possible to resolve the differences between these five studies nor is it easy to argue that one study has more weight on this question than any other. The studies use different measures of exposure or time since exposure, are done on different populations and have different statistical power to detect a trend.

In rodent carcinogenicity studies, there is clear evidence of a biological gradient.

In general, there is support that a biological gradient exists for the epidemiological data and thus support from this aspect of the Bradford-Hill evaluation.

VIII. Temporal Relationship

Exposure must come before the cancers occur otherwise the epidemiology studies are useless. In this case, it is clear that exposure came before the onset of NHL. **The need for a temporal relationship in the data supporting a causal association between glyphosate and NHL is satisfied.**

IX. Specificity

There are other causes of NHL[76-79] so this group of cancers is not specific to glyphosate. Glyphosate has been studied in multiple population for multiple types of tumors; the only class of cancers consistently seen as positive for exposure to glyphosate is NHL. **There is strong support for specificity**.

X. Coherence

There is strong support for coherence in the data supporting a causal association of glyphosate and glyphosate formulations with NHL.

Humans, coming into contact with glyphosate in multiple ways, can absorb the

compound into their bodies where it has been measured in blood and in urine. In laboratory animals, and to some degree in humans, absorption, distribution and elimination of glyphosate and glyphosate compounds have been studied and show that glyphosate gets into the mammals' bodies, distributes to numerous organs and is eliminated in urine. The animal cancer studies clearly demonstrate that glyphosate in mammals can have toxic effects.

Mouse models have long served as surrogates for humans in understanding and developing treatments for many diseases. The same holds true for lymphoid tumors seen in humans. For over 30 years, mouse models have been studied and evaluated as surrogates for NHL[80-84]. These publications and the associated classification systems for humans and mice indicate a close linkage between the diseases in humans and mice. Thus, coherence is supported by the increased risk of malignant lymphomas in CD-1 mice, the marginal increase in these tumors in Swiss mice and the strong similarity between malignant lymphomas in mice and NHL in humans.

Glyphosate significantly (p not given) reduced survival in a mouse model of multiple myeloma (Vk* MYC) after 72 weeks of exposure relative to the glyphosate-free controls [85]. The treated mice had significantly increased spleen weight ($p<0.05$) and splenocyte counts ($p<0.05$). The treated mice also demonstrated histological disorganization and overall toxicity. Wild-type (WT) littermates (they contain one copy of the Vk*MYC gene rather than two) also demonstrated toxicity and disorganization, but to a lesser degree. Glyphosate induced splenomegaly in both WT and Vk*MYC mice. Both WT and Vk*MYC mice demonstrated a significant increase ($p<0.05$) in IgG levels when compared to controls. Vk*MYC treated mice had a clear M-spike, WT mice had a weaker M-spike and no M-spike was detected in untreated animals regardless of genetics.

Activation-induced cytidine deaminase (AID, a marker of MGUS induction) was upregulated in both bone marrow and spleen of both Vk*MYC and WT mice in the 72-week study. The same upregulation in the spleen and bone marrow were seen in the 7-day exposure animals in a dose-dependent fashion with significant pairwise increases at 10 and 30 g/L. A smaller dose-dependent increase was seen in lymph nodes but this was still significantly different from controls at the highest exposure 30g/L. This upregulation of AID support an AID-mediated mutational mechanism for the induction of MM in these mice.

There is strong support for coherence in the data supporting a causal association of glyphosate and glyphosate formulations with NHL.

XI. Experimental Evidence in Humans

There is no experimental evidence in humans since purposely exposing humans to a pesticide, especially one that is probably carcinogenic, is not ethical and would never pass review by a human subject's advisory board.

XII. Analogy

I am unaware of any analogous compounds from the scientific literature. This, however, is not an area where I have sufficient background to express an opinion.

XIII. Summary of Bradford Hill Evaluation

Glyphosate probably causes NHL and, given the human, animal and experimental evidence, I assert that, to a reasonable degree of scientific certainty, the probability that glyphosate causes NHL is high.

Table ES- 3 summarizes the information for each of Hill's aspects of causality.

Causality is strengthened because the better epidemiological studies show a **consistent** positive association between cancer and the exposure. In answer to Hill's question, the relationship between NHL and glyphosate exposure has been observed by different persons, in different places, circumstances, and times.

Causality is strengthened because the **strength of the observed associations**, when evaluated simultaneously, are statistically significant, the findings are uni-directional and the results are unlikely to be due to chance. Even though none of the individual studies provide relative risks or odds ratios that are large and precise, the meta-analyses have objectively shown that the observed association across these studies is significant and supports a positive association between NHL and glyphosate.

Biological plausibility is strongly supported by the animal carcinogenicity data and the mechanistic data on genotoxicity and oxidative stress. When addressing biological plausibility, the first question generally asked is "Can you show that glyphosate causes cancers in experimental animals?" In this case, the answer to that question is clearly yes. Glyphosate has been demonstrated to cause cancer in two strains of rats and one strain of mice.

Table ES- 3: Summary conclusions for Hill's nine aspects of epidemiological data and related science

Aspect	Conclusion	Reason
Consistency of the observed association	Strong	Multiple studies, most are positive, meta-analysis shows little heterogeneity and positive findings, different research teams, different continents, different questionnaires, no obvious bias or confounding
Strength of the observed association	Strong	Seven core epidemiology studies all show the same modest increase, significant meta-analyses
Biological plausibility	Very Strong	Multiple cancers in multiple species, not due to chance, increased risk of rare tumors, convincing evidence for genotoxicity and oxidative stress

Biological gradient	Moderate	Clearly seen in the two case-control studies and a pooled analysis that evaluated it, not seen in the cohort study
Temporal relationship of the observed association	Satisfied	Exposure clearly came before cancers
Specificity of the observed association	Strong	The only cancers linked to glyphosate are NHL and its subtypes
Coherence	Strong	Glyphosate is absorbed, distributed and excreted from the body, cancers seen in the mice have strong similarity to human NHL and transgenic animals support a brifge between findings in the humans and the mice
Evidence from human experimentation	No data	No studies are available
Analogy	No data	No studies available in the literature

The next question generally asked is "Does the mechanism by which glyphosate causes cancer in experimental animals also work in humans?" The best understood mechanism by which chemicals cause cancer in both humans and animals is through damaging DNA that leads to mutations in cells that then leads to uncontrolled cellular replication and eventually cancer. It is absolutely clear from the available scientific data that both glyphosate and glyphosate formulations are genotoxic. This has been amply demonstrated in humans that were exposed to glyphosate, in human cells *in vitro*, in experimental animal models and their cells *in vitro* and *in vivo*, and in wildlife. One way in which DNA can be damaged is through the presence of free oxygen radicals that overwhelm a cell's antioxidant defenses. Glyphosate induces this type of oxidative stress, providing additional support for a biological mechanism that works in humans.

In general, there is support that a **biological gradient** exists for the epidemiological data and thus support from this aspect of the Bradford-Hill evaluation. Glyphosate ORs increased with time since first exposure and with intensity of use per year in the three related case-control studies that evaluated at least one of these issues.

There is clearly the proper **temporal relationship** with the exposure coming before the cancers.

The human evidence is **coherent**. The basic findings in humans agree with the animal evidence for absorption, distribution and elimination of glyphosate. Also, one of the tumors seen in mice has almost the same etiology as NHL and transgenic animals prone to get B-cell tumors through a mechanism linked to humans show increased risk after exposure to glyphosate.

NHL is not specific to glyphosate exposure however, glyphosate is **specific** to NHL. There is no **experimental evidence** in humans and I did not find any references where researchers looked for **analogous** compounds with similar toxicity.

Hill (1965)[4] asks "*is there any other way of explaining the set of facts before us, is there any other answer equally, or more, likely than cause and effect?*" There is no

better way of explaining the scientific evidence relating glyphosate to an increase in NHL in humans than cause and effect.

In my opinion, glyphosate probably causes NHL and, given the human, animal and experimental evidence, I assert that, to a reasonable degree of scientific certainty, the probability that glyphosate causes NHL is high.

1. Charge

Glyphosate acid is a colorless, odorless, crystalline solid. Glyphosate is the term used to describe the salt that is formulated by combining the deprotonated glyphosate acid and a cation (isopropylamine, ammonium, or sodium). This expert report is intended to review the available scientific evidence relating to the potential of glyphosate and glyphosate-based formulations (GBFs), including Roundup®, to cause Non-Hodgkin's Lymphoma (NHL) in humans.

2. Qualifications

I received an undergraduate degree in mathematics in 1977 from Nicholls State University and a Master's degree and Ph.D. in biostatistics from the University of North Carolina School of Public Health in 1979 and 1981 respectively. My Ph.D. thesis addressed the optimal way to design a two-year rodent carcinogenicity study to assess the ability of a chemical to cause cancer[86, 87]; the optimal dosing pattern from my thesis is still used by most researchers. My first employment following my doctoral degree was a joint appointment at the National Institute of Environmental Health Sciences (NIEHS) and the National Toxicology Program (NTP) to conduct research on the design and analysis of experiments generally employed in toxicology. After 5 years with NIEHS/NTP, I developed my own research group which eventually became the Laboratory of Quantitative and Computational Biology and then the Laboratory of Computational Biology and Risk Assessment (LCBRA). One highlight during this period was the development of the Poly-3 Test for survival adjustment of data from two-year carcinogenicity studies in rodents[88, 89]; this test is used as the main method of analysis of these studies by the NTP and many others. We also did a complete analysis of the historical controls animals from the NTP studies[90, 91]. The LCBRA focused on the application of computational tools to identify chemicals that are toxic to humans, to develop tools for understanding the mechanisms underlying those toxicities and to quantify the risks to humans associated with these toxicities. The main toxicological focus of the LCBRA was cancer and my laboratory developed many methods for applying multistage models to animal cancer data and implemented the use of these models in several experimental settings[92-104]. In my last few years at the NIEHS/NTP, my research focus expanded to the development of tools for evaluating the response of complex experimental and human systems to chemicals[105-109] and the name of the laboratory shifted to Environmental Systems Biology.

Over my 32 years with the NIEHS/NTP, I was involved in numerous national priority issues that went beyond my individual research activities. After Congress asked NIEHS to work with the Vietnamese government to address the hazards associated with Agent Orange use during the Vietnamese War, I was given the responsibility of working with my counterparts in Vietnam to build a research program in this area[110]. Congress also tasked NIEHS with developing a research program (EMF-RAPID) to address concerns about the risks to humans from exposure to power lines and to report back to Congress

on what we found. I was in charge of evaluating all research developed under this program and was responsible for the final recommendations to Congress on this issue[111-113].

While at the NIEHS/NTP, I also had administrative positions that relate to my qualifications. From 2000 to 2006 I was the Director of the Environmental Toxicology Program (ETP) at NIEHS. The ETP included all of the toxicology research laboratories within the NIEHS Intramural Research Program. It was my responsibility to ensure the research being done was pertinent to the mission of the NIEHS, addressing high priority concerns about toxic substances and human health and that the NIEHS had adequate resources to complete this research.

During this time I was also Associate Director of the NTP, a position in which I was the scientific and administrative director of the NTP (The Director of the NTP was also the NIEHS Director and gave me complete autonomy in the management and science of the NTP). These two positions were historically always combined at the NIEHS and the NTP so that one person was in charge of all toxicological research at the NIEHS/NTP. The NTP is the world's largest toxicology program, routinely having 15 to 25 active two-year carcinogenicity studies, numerous genetic toxicology studies and many other toxicological studies being conducted at any given time. The NTP two-year carcinogenicity studies and their technical reports are also considered the "gold standard" of cancer studies due to their extreme high quality, their tremendous utility in evaluating human health hazards and the rigor and transparency they bring to the evaluation of the data. All data from NTP two-year cancer studies are publicly available including data on individual animals and images from the pathology review of each animal. The NTP is also home to the Report on Carcinogens, the US Department of Health and Human Services official list of what is known or reasonably anticipated to be carcinogenic to humans. It was my responsibility to decide what items eventually went onto this list while I was Associate Director of the NTP. In 2006, I became an Associate Director of the NIEHS, a senior advisor to the director and the director of the Office of Risk Assessment Research (ORAR). ORAR focused on stimulating new research areas on the evaluation of health risks from the environment and addressed major risk assessment issues on behalf of the NIEHS/NTP. For example, in this capacity, I lead a multiagency effort to understand the health risks to humans from climate change and to develop a research program in this area[114].

I left the NIEHS/NTP in 2010 to become the Director of the National Center for Environmental Health (NCEH) at the Centers for Disease Control and Prevention and simultaneously Director of the Agency for Toxic Substances and Disease Registry (ATSDR). NCEH does research and supports activities aimed at reducing the impact of environmental hazards on public health. One well-respected research effort of the NCEH is the National Biomonitoring Program. This program tests for the presence of hundreds of chemicals in human blood and urine in a national sample of people in the United States. ATSDR advices the Environmental Protection Agency (EPA) and communities on the potential health impacts from toxic waste dump sites (superfund sites). ATSDR is required by law to produce ToxProfiles. These are comprehensive

reviews of the scientific literature for specific chemicals generally found at superfund sites. They also provide an assessment of the safety of these chemicals. As part of my activities at ATSDR, I began a modernization of the ToxProfiles to use systematic review methods in their assessments; this effort was linked to a similar effort that I had helped to implement at the NIEHS/NTP.

Aside from my official duties in my various federal jobs, I also served on numerous national and international science advisory panels. Most notable, for my qualifications for this statement, are my serving as Chair from 2005 to 2010 of the Subcommittee on Toxics and Risk of the President's National Science and Technology Council, member and chair of EPA'S Science Advisory Panel from 1998 to 2003 (focused specifically on advising their pesticides program) and chair of the International Agency for Research on Cancer (IARC) advisory group that updated and improved its rules for reviewing scientific data to ensure that conclusions on the carcinogenicity of human exposures are the best possible (Preamble)[6]. As part of my work on science advisory panels, I have served on EPA's Science Advisory Board, as an advisor to the Australian Health Council on risk assessment methods, as an advisor to the Korean Food and Drug Administration on toxicological methods, and served on several World Health Organization (WHO) International Program on Chemical Safety scientific panels dealing with risk assessment. Besides the guidelines for evaluating cancer hazards used by the IARC, I have either chaired or served as a member of scientific panels developing guidance documents for other organizations including the EPA.

I have received numerous awards, most notably the Outstanding Practitioner Award from the International Society for Risk Analysis and the Paper of the Year Award (twice) from the Society of Toxicology Risk Assessment Specialty Section. I am a fellow of the American Statistical Association, the International Statistical Institute, the World Innovation Foundation and the Ramazinni Institute. I have published over 250 peer-reviewed scientific papers, book chapters and technical documents on topics in toxicology and risk assessment.

Finally, I have served on numerous national and international committees tasked with evaluating the risk and/or hazard of specific environmental chemicals, including glyphosate. For example, I have contributed to risk assessments for EPA, the Food and Drug Administration, the Centers for Disease Control and Prevention, the National Institutes of Health, the WHO and IARC.

3. Explanation of Bradford Hill Causality Evaluation

Most of the guidelines [1-3] used for cancer risk assessment trace their origins to a paper by Hill (1965) [4]. The IARC review of glyphosate [5] followed guidelines derived from Hill (1965) and concluded glyphosate was "probably carcinogenic to humans".

The evaluation of whether glyphosate and/or GBFs can cause NHL in humans requires the review and synthesis of scientific evidence from studies of human populations (epidemiology), animal cancer studies, and studies investigating the mechanisms through which chemicals cause cancer. Many different approaches[115, 116] are used

to synthesize these three areas of science to answer the question "Does this chemical cause cancer in humans?" In any of these three science areas, the quality of the individual studies has to be assessed and summarized to make certain the studies included in the overall assessment are done appropriately. Once the quality of the individual studies has been assessed, a judgment needs to be made concerning the degree to which the studies support a finding of cancer in humans. To do this, the EPA, IARC, the European Chemical Agency (EChA), the US Report on Carcinogens, and many others use guidelines[1, 3, 6, 117] that rely upon aspects of the criteria for causality developed by **Hill (1965)**[4].

Hill listed nine (9) aspects of epidemiological studies and the related science that one should consider in assessing causality. The presence or absence of any of these aspects is neither sufficient nor necessary for drawing inferences of causality. Instead, the nine aspects serve as means to answer the question of whether other explanations are more credible than a causal inference. As noted by Hill:

> *"None of my nine viewpoints can bring indisputable evidence for or against the cause-and-effect hypothesis and none can be required as a sine qua non. What they can do, with greater or less strength, is to help us to make up our minds on the fundamental question — is there any other way of explaining the set of facts before us, is there any other answer equally, or more, likely than cause and effect?"*

The nine aspects cited by Hill include consistency of the observed association, strength of the observed association, biological plausibility, biological gradient, temporal relationship of the observed association, specificity of the observed association, coherence, evidence from human experimentation and analogy. These are briefly described below.

An inference of causality is strengthened when several of the studies show a **consistent positive association** between cancer and the exposure. This addresses the key issue of replication of studies which is critical in most scientific debates. If studies are discordant, differences in study quality, potential confounding, potential bias and statistical power are considered to better understand that discordance.

An inference of causality is strengthened when the **strength of the observed association** in several studies are large and precise. These large, precise associations lessen the possibility that the observed associations are due to chance or bias. A small increase in risk of getting cancer does not preclude a causal inference since issues such as potency and exposure level may reduce the ability of a study to identify larger risks. Meta-analyses provide an objective evaluation of the strength of the observed association across several studies with modest risks to help clarify strength of the observed associations.

An inference of causality is strengthened when there is data supporting **biological plausibility** demonstrated through experimental evidence. Animal carcinogenicity studies, in which tumor incidence is evaluated in experimental animals exposed to pure

glyphosate, play a major role in establishing biological plausibility. There are numerous types of mechanisms that can lead to cancer[75], most of which can be demonstrated through experimental studies in animals, human cells, animal cells, and/or other experimental systems. Occasionally, occupational, accidental or unintended exposures to humans allow researchers to evaluate mechanisms using direct human evidence.

An inference of causality is strengthened when there is a **biological gradient** showing a reasonable pattern of changing risk with changes in exposure (e.g. risk increases with increasing exposure or with longer exposure). In many epidemiological studies, this aspect cannot be examined due to limitations in the study design or due to a lack of clarity in the presentation of the results. When a study does address an exposure-response relationship, failure to find a relationship can be due to a small range of exposures, insufficient sample size or a changing exposure magnitude over time that has not been accounted for.

An inference of causality is strengthened when there is a **temporal relationship** in which the exposure comes before the cancer. This aspect is necessary to show causality; if it is not present, a causal inference is not plausible. Because the latency period for cancers can be long (years), evaluation of studies should consider whether the exposure occurred sufficiently long ago to be associated with cancer development.

An inference of causality is strengthened when the exposure is **specific** for a given cancer. This would mean that the disease endpoint being studied is only due to the cause being assessed or that, even though many different cancers have been studied for an association with a given exposure, only one type of cancer shows a consistent association.

An inference of causality is strengthened when other lines of experimental evidence are **coherent** with a causal interpretation of the association seen in the epidemiological evidence. To evaluate coherence, information from animal carcinogenicity studies, mechanistic investigations and information on the metabolism of the chemical being studied would be considered.

An inference of causality is strengthened when there is **experimental evidence in humans** supporting a causal interpretation. Seldom is this type of information available when addressing the toxicity of chemicals. However, experiments in which an individual reduces or limits exposures and the risk of cancer is reduced would carry considerable weight in the evaluation (e.g. studies evaluating the cancer risks of people who stop cigarette smoking compared with continuing smoking have demonstrated reduced lung cancer risks). No such data are available for glyphosate.

Finally, an inference of causality is strengthened when there are other chemical agents with **analogous** structures showing similar effects in humans and/or animals and/or showing similar biological impacts in mechanistic studies.

The most logical approach to developing an inference of causality is to step through each of the aspects of causality developed **by Hill (1965**)[4] and apply them to the

available data for glyphosate and for glyphosate formulations. This is done in the sections that follow.

4. Human Epidemiological Studies of NHL

There are seven epidemiology studies done on two different continents by different research groups using different designs, questionnaires and study populations that show consistent positive responses with no obvious bias or confounding that would explain the results. There are two additional studies that have problems with exposure misclassification and differ from the other seven in response.

4.1 Search Criteria

In their meta-analysis, **Chang and Delzell (2016)**[7] performed a systematic literature search of all scientific literature up to June, 2015, to identify all epidemiological studies that were pertinent to evaluating an association between glyphosate and NHL. They identified 12 relevant epidemiology studies[8, 11-18, 20, 118, 119]. Their search string for PubMed is as follows:

(glyphosat_ OR glifosat_ OR glyfosat_ OR gliphosat_OR Roundup OR Round-up OR 1071-83-6 OR 38641-94-0 OR 70901-12-1 OR 39600-42-5 OR 69200-57-3 OR 34494-04-7 OR 114370-14-8 OR 40465-66-5 OR 69254-40-6 OR (aminomethyl w phosphonic_) OR 1066-51-9 OR pesticid_ OR herbicid_ OR organophosphorus compounds [MeSH] OR pesticides [MeSH] OR herbicides [MeSH]) AND (leukemi_ OR leukaemi_ OR lymphoma_ OR NHL OR lymphopoietic OR hemato_ OR hematopoie_ or hematolog_ OR lymphoid OR myeloid OR myeloma OR leukemia [MeSH] OR lymphoma [MeSH] OR multiple myeloma [MeSH]) AND (cases OR controls OR case-control OR cohort)

Their search agreed with all reviews of glyphosate through 2016 and I will use their findings from the literature up until 2015. To cover from June 2015 to the present (May 11, 2020), I used their searching algorithm and identified 735 additional published studies, four of which were new epidemiology studies or pooled analyses or meta-analyses directly related to this review [19, 21, 23, 27] and numerous studies that have bearing on the interpretation of these and previous epidemiology studies. These 17 studies will be considered for use in this evaluation. I will focus on using the results of these studies in evaluating causality so I will only briefly describe each study.

4.2 Case-Control Studies

Cantor et al. (1992)[8] did an in-person interview study comparing 622 white men, newly diagnosed with NHL, to 1245 population-based controls in Iowa and Minnesota. They originally identified 780 cases, of which 694 (89%) were interviewed. After pathology review, only 622 were found to have NHL, the remaining cases having leukemia or other diseases. Three different sources of controls were used, random digit dialing (76.7% response rate), Health Care Financing Administration rolls (79% response rate) and deceased controls with eligible proxies (77% response rate). Both cases and controls were questioned regarding their use of agricultural products including

Roundup® and any other glyphosate-based formulations. For deceased or incompetent controls (184) and cases (number not given), proxy interviews were done with a close relative. When cases in farmers were compared to cases in non-farmer controls, 26 cases (out of 266) and 49 controls (out of 547) had handled herbicides containing glyphosate yielding an odds ratio[5] (OR) of 1.1 (95% confidence interval 0.7-1.9). This analysis controlled for vital status, age, state, cigarette smoking status, family history of lymphopoietic cancer, high-risk occupations and high-risk exposures in a logistic analysis. The authors noted there was "minimal evidence for confounding of results for any single pesticide by exposure to pesticides belonging to other chemical families." Because the exposure is determined based on interviews in cases and controls, this study has the potential for recall bias[6]. However, the authors note that the bias could both increase or decrease the OR because of non-differential exposure misclassification[7] because of difficulties in accurate recall of past pesticide exposures for both controls and treated individuals. This study will not be included separately into the evaluation since it overlaps with **De Roos et al. (2003)**[11] and **Pahwa et al. (2019)** [19]

Two additional studies conducted by **Zahm et al. (1990)**[10] in Nebraska and **Hoar et al. (1986)**[9] in Kansas collected information on pesticide and herbicide use, but did not report specifically on the effects of glyphosate. **De Roos et al. (2003)**[11] pooled the data from these two studies with the data from **Cantor et al. (1992)**[8] to examine pesticide exposure to glyphosate in farming as risk factors for NHL. The three case-control studies[8-10] had slightly different designs. The design for the Minnesota study[8] is provided directly above. In Nebraska[10], the cases were identified through the Nebraska Lymphoma Study Group and area hospitals for 66 counties and included all white men and women diagnosed with NHL between July 1, 1983 and June 30, 1986. Controls were obtained by random-digit dialing, Medicare records or state mortality files depending upon age and vital status. All study participants were over age 21 and even though this study included a few women, they were excluded from the **De Roos et al. (2003)** analysis. The response rates for cases and controls were 91% and 87% respectively. In Kansas[9], cases were randomly sampled from a registry at the University of Kansas of white men, over age 21, diagnosed between 1979 and 1981. The response rates for cases and controls were 96% and 94% respectively. Controls were population-based matched on age and vital status. As for the Nebraska study, controls

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5 The odds ratio (OR) is calculated as the proportion of exposed cases with disease to exposed controls divided by the proportion of non-exposed cases to non-exposed controls. For rare diseases, this value approximates the population risk ratio (PRR) which is the probability of having the disease in exposed individuals divided by the probability of having the disease in non-exposed individuals. If the PRR is 1, then there is no difference in the probability of having the disease regardless of your exposure. Values of PRR greater than 1 imply the risk is higher in the exposed population. Because the OR is an estimate of the PRR for rare diseases, it is usually accompanied by a 95% confidence interval that describes the probable range of the estimate. If the OR is greater than 1, then the exposure is associated with the disease. If the lower 95% confidence bound for the OR is greater than 1, this is typically used to say the association is statistically significant.

6 Recall bias occurs when cases are more likely to say they are exposed to glyphosate than controls or when controls are more likely to say they are exposed to glyphosate than cases. The recall must be different for the cases than the controls for this to cause a bias; errors in recalling past exposures that happen for both cases and controls would not be recall bias.

7 Non-differential exposure misclassification occurs when the probability of an error in determining whether an individual is exposed or not is the same for both cases and controls.

for live cases were obtained from Medicare records for cases 65+ and by random-digit dialing for cases <65 years; controls for deceased patients came from state mortality records. The resulting pooled case-control study had 870 cases and 2569 controls (for analyzing the relationship between glyphosate and NHL, there were only 650 cases and 1933 controls following exclusion of subjects with missing data). For any glyphosate exposure, there were 36 exposed cases and 61 exposed controls with an OR (95% confidence interval) of 2.1 (1.1-4.0) in a logistic regression analysis controlling for all other pesticides reported, age and study site. The authors also analyzed the data using a Bayesian hierarchical regression analysis yielding an OR (95% confidence interval) of 1.6 (0.9-2.8) controlling for the same parameters as the logistic regression. They also conducted an analysis of "potentially carcinogenic" pesticides which included glyphosate. When just one of these pesticides was used by subjects, the logistic regression OR was 1.6 (0.8-3.1), two to four pesticides yielded an OR of 2.7 (0.7 to 10.8) and when more than five were used, the OR was 25.9 (1.5-450.2) in the logistic regression analysis and 1.1 (0.8-1.7), 1.3 (0.7-2.3) and 2.0 (0.8-5.2) respectively for the Bayesian analysis. Removing glyphosate from the list of "potentially carcinogenic" pesticides yielded equivalent ORs of 1.2 for one pesticide, 1.2 for two to four pesticides and 1.1 for five or more pesticides. The authors note that the positive results seen in their study are not likely due to recall bias since there were few associations seen over the 47 pesticides they studied. Also, although some of the positive results could be due to chance, the use of the hierarchical regression analysis theoretically decreases the chance of false positive findings. In the Kansas study[9], suppliers for 110 subjects with farming experience were identified and provided information on the subjects' crops and pesticide purchases. In general, the suppliers reported less pesticide use than the subjects of the study with no consistent differences in agreement rates between cases and controls. The agreement between suppliers and subjects improved when pesticide use during the last 10 years was considered. This supports a reduced role of recall bias in these studies and a possible role of non-differential exposure misclassification. The reduced ORs when using the Bayesian analysis as compared to the logistic regression is not surprising because the authors used a somewhat non-informative prior rather than a less conservative prior. In addition, adjustment for 47 pesticides is also likely to reduce the significance of the observed ORs for pesticides that are associated with NHL as demonstrated by the analysis of "potentially carcinogenic" pesticides (this model is possibly over-parameterized since it includes over 47 dependent variables for only 36 exposed cases; this can significantly reduce the ORs and increase the confidence bounds). This pooled case-control study will be included in the evaluation of causation.

Lee et al. (2004)[118] pooled data from **Zahm et al. (1990)**[10] and **Cantor et al. (1992)**[8] (previously described) to evaluate whether asthma acts as an effect modifier of the association between glyphosate exposure and NHL. Women were included in this analysis whereas **De Roos et al. (2003**)[11] excluded women. The final study published by Lee included 872 cases and 2336 controls of which 45 cases and 132 controls had been told by their doctors they had asthma. The OR of association between glyphosate and NHL in non-asthmatics was 1.4 (0.98-2.1) and 1.2 (0.4-3.3) in asthmatics when controlling for age, vital status and state (geographical location). This study completely

overlaps with the study by **De Roos et al. (2003)**[11] with the exception of the inclusion of the few women in the study by **Zahm et al. (1990)**[10]. Since this study only looks at effect modification due to asthma, it does not contribute to the overall evaluation of causality and it will be excluded from further evaluations.

Nordstrom et al. (1998)[12] conducted a population-based case-control study of hairy cell leukemia (HCL); a subtype of B-cell NHL) in Sweden that included an evaluation of exposures to glyphosate. The study included 111 men with NHL reported to the Swedish Cancer Registry between 1987 and 1992 (with one patient from 1993 accidentally included). Controls (400 in total) were drawn from the National Population Registry matched for age and county with the cases. The response rates were 91% for cases (10 refused to participate out of the original 121) and 83% for controls (84 controls refused to participate out of 484 selected). Almost all questionnaires were answered by the subject of the study (4 cases and 5 controls were answered by proxies). The study reported an OR for glyphosate exposure and HCL of 3.1 (0.8-12) controlling only for age. This study had very limited power for detecting an association because there were only four cases and five controls with glyphosate exposure (1.8% of the total study population). In addition, because they failed to adjust for other exposures, the potential for confounding in this study is greater than those presented previously. The authors noted that they attempted to minimize recall bias by only using living cases in the analysis. Also, even though matching was performed to identify the controls, this matching was not used in the final analysis. This study was later used in a pooled analysis of HCL and NHL[14] and will not be considered independently in the evaluation for causation but will be used in the context of the pooled analysis.

Hardell and Eriksson (1999)[13] conducted a population-based case-control study of all male patients older than 25 years diagnosed with NHL between 1987 and 1990 in the four most northern counties of Sweden. After excluding misdiagnosed cases, they included 442 cases of which 404 answered their questionnaire (most by proxy) for a response rate of 91%; 192 of these cases were deceased. For each living case, two male matched controls were chosen from the National Population Registry and matched on age and county. For each deceased case, two male controls were chosen from the National Registry for Causes of Death, matched for age and year of death. The response rate for the controls was 84% (741 out of 884 identified). Study subjects were sent a detailed questionnaire and, in most cases, this was supplemented with a phone interview. A complete working history was obtained with questions regarding exposure to numerous chemicals to avoid a focus on pesticides and organic solvents, the focus of the study. Exposure was defined as at least one full day of exposure more than one year before diagnosis. For glyphosate exposure, the authors identified four cases and three controls with exposures and a univariate OR of 2.3 (0.4-13). A multivariate analysis of both glyphosate and phenoxy herbicides produced an OR of 5.8 (0.6-54). The study has limited power for detecting an effect because the exposure frequency is very low (0.6% exposed). This study was later used in a pooled analysis of HCL and NHL[14] and will not be considered independently in the evaluation for causation but will be used in the context of the pooled analysis.

Hardell et al. (2002)[14] conducted a pooled analysis of NHL and HCL by combining the studies of **Nordstrom et al. (1998)[12]** and **Hardell and Eriksson (1999)**[13]. This study fully overlaps with the previous two studies. The analysis controlling for age, study, county and vital status yielded an OR of 3.04 (1.08-8.52) based on eight exposed cases and eight exposed controls. A more extensive analysis additionally controlled for other pesticides and yielded a smaller OR of 1.85 (0.55-6.20). As for the study by **De Roos et al. (2003**), the analysis may be over-parameterized (more than eight dependent variables with only eight exposed cases) which could lead to a reduction in the ORs and larger confidence bounds. Even with the pooled data, **Hardell et al. (2002)** had limited power to detect an effect because the exposure frequency for cases and controls was very low (1% exposed). This study is a valid case-control study and will be used in the evaluation of causality.

In a later study, **Eriksson et al. (2008)**[15] conducted a population-based case-control study where cases were identified as NHL patients aged 18-74 years diagnosed in four major hospitals in Sweden from December 1, 1999 until April 30, 2002. In total, 995 cases were identified as matching the study parameters with 910 (91%) answering the questionnaire shortly after diagnosis. All cases were classified into subgroups with 810 B-cell, 53 T-cell, and 38 unspecified lymphomas. Controls (1,108) were randomly selected from the population registry and matched on health service, region, sex and age and interviewed in several periods during the conduct of the study; 1,016 controls responded to the questionnaire (92% response rate). Study subjects were sent a detailed questionnaire and, in many cases, a phone interview followed. Exposure was defined as at least one full day of exposure more than one year before diagnosis. The univariate analysis, adjusting for age, sex and year of diagnosis (cases) or enrollment (control) yielded an OR of 2.02 (1.10-3.71) based on 29 exposed cases and 18 exposed controls. When cases and controls were divided into those with ≤10 days per year exposure and those with >10 days per year exposure, the ORs were 1.69 (0.70-4.07) and 2.36 (1.04-5.37) respectively. When diagnoses were grouped into various subtypes of NHL, the results did not change dramatically except for small lymphocytic lymphoma and chronic lymphocytic lymphoma which showed an increased OR of 3.35 (1.42-7.89). A multivariate analysis of glyphosate controlling for other agents with statistically increased odds ratios and/or odds ratios greater than 1.5 yielded an OR of 1.51 (0.77-2.94). In a similar analysis to the multivariate analysis, latency periods of one to ten years showed an OR of 1.11 (0.24-5.08) and >10 years had an OR of 2.26 (1.16-4.40). This study was much larger than the previous Swedish studies (2.3% exposed) and, although there may have been confounding from other pesticides, this was addressed in the multivariate analysis and the latency analysis. This study is a valid case-control study and will be used in the evaluation of causality.

McDuffie et al. (2001)[16] recruited incidence cases of NHL in men 19 years or older from six Canadian provinces with a first diagnosis between September 1, 1991 and December 31, 1994. Each provincial Cancer Registry or, in the case of Quebec, hospital, had a target number of cases and ended recruitment when the case number was reached. Controls were men 19 years or older selected at random from provincial health insurance records, computerized telephone listings or voter registration lists,

depending upon the province. Cases and controls were sent questionnaires with surrogates ineligible to answer the questionnaires for deceased cases or controls. Each subject who reported 10 hours per year or more of pesticide exposure and a random sample of 15% who reported less exposure were interviewed by telephone to obtain details on pesticide use. A pilot study was conducted to obtain an improved version of the telephone interview questionnaire used by **Hoar et al. (1986)**[9] and **Zahm et al. (1990)**[10] that would provide accurate pesticide exposure assessment in the form of a screening questionnaire and a telephone interview questionnaire. This was followed by a validation study (27 farmers) where the final questionnaires used to screen and include potential cases and controls were administered and the answers regarding pesticide usage showed excellent concordance with purchases through their local agrochemical supplier. The screening questionnaire was returned by 517 cases of NHL (67.1% response rate) and 1506 controls (48% response rate). Following analysis of the screening questionnaire, the telephone interview was administered to 179 cases and 456 controls to obtain more detailed exposure information. The OR for glyphosate exposure and NHL was 1.26 (0.87-1.80) stratified by age group and province of residence and the OR was 1.20 (0.83-1.74) when the analysis also controlled for significant medical variables (51 exposed cases and 133 exposed controls). An exposure-response evaluation was performed where the OR for exposure between zero to two days per year was 1.0 (0.63-1.57) and for greater than two days per year was 2.12 (1.20-3.73) with the latter group having 23 exposed cases and 36 exposed controls. This study had excellent sample size and power (8.1% of subjects exposed), but a low response rate to the screening questionnaire. Also, by adjusting for significant medical variables, this study ruled out many confounders but did not adjust for other pesticide exposures. The effort to validate the recall of pesticide usage for farmers supports a lack of recall bias in the study. This study is a valid case-control study and will be used in the evaluation of causality.

Hohenadel et al. (2011)[119] re-analyzed the data of **McDuffie et al. (2001)**[16] to specifically investigate the impact of exposure to multiple pesticides on NHL. Four cases of NHL were excluded from this evaluation following a pathology review. They reported associations with the use of glyphosate with and without malathion but not with glyphosate overall. The OR for glyphosate (ever used) without malathion (ever used) was 0.92 (0.54-1.55) and the OR for glyphosate (ever used) with malathion (ever used) was 2.1 (1.31-3.37). **Chang and Delzell (2016)**[7] combined the ORs from the glyphosate only analysis with the glyphosate and malathion analyses using random-effects meta-analysis to get a combined OR for glyphosate of 1.4 (0.62-3.15). This study was specifically targeted to interactions of various pesticides and does not substantively contribute to an evaluation of glyphosate. Since it is a refined analysis of **McDuffie et al. (2001)**[16], it will be included in the evaluation of causation only in the context of the combined analysis provided by **Chang and Delzell (2016).**

Orsi et al. (2009)[17] conducted a hospital-based case-control study of men and women diagnosed with lymphoid neoplasms in five hospitals in France between 2000 and 2004 who were aged 20-75 years (the abstract gives the age range as 18-75 years). All diagnoses were cytologically or histologically confirmed. The evaluation only included

men and questionnaires/interviews were completed by 491 cases (95.7% response rate) which included 244 cases with NHL. Controls were patients in the same hospital (mostly orthopedic or rheumatological patients) with no prior history of lymphoid neoplasms and excluding patients admitted to the hospital for cancer or a disease directly related to occupation, smoking or alcohol abuse. The controls were matched to cases by hospital and age. Of the 501 candidate controls, 456 participated (91% response). Exposure was evaluated differently for subjects who had non-occupational exposures from those who had occupational exposures. For both, the subjects had to fill out a questionnaire/interview on occupations and home gardening pesticide exposures. For those who had worked professionally as farmers or gardeners for at least 6 months, a specific agricultural occupational questionnaire/interview was administered and exposure was determined on the basis of this extra data. The OR for occupational use of glyphosate and NHL was 1.0 (0.5-2.2) with 12 exposed cases and 24 exposed controls stratified by age and center category. A further analysis was done by individual subtypes of NHL with an OR of 1.0 (0.3-2.7) for diffuse large cell lymphoma, 1.4 (0.4-5.2) for follicular lymphoma, 0.4 (0.1-1.8) for chronic lymphocytic leukemia (CLL) and 1.8 (0.3-9.3) for HCL. No separate analysis of non-occupational use of glyphosate was provided, nor does it seem specific data on glyphosate usage was ascertained for subjects who were not professional farmers or gardeners. This could lead to non-differential misclassification of exposure which could reduce the ORs of the study. Barring this, the sample size was sufficient to detect an effect (5.3% with occupational exposure) and this study will be included in the evaluation of causality.

Cocco et al. (2013)[18] evaluated data from a multi-center case-control study of lymphoid neoplasms in six European countries from 1998 to 2004. Cases included only adult patients diagnosed with lymphoma during the study period drawn from participating centers. Controls were either selected by sampling from the general population on sex, age group, and residence area (Germany, Italy), or from hospital controls matched to the patient excluding patients with cancer, infectious diseases, and immunodeficiency diseases (Czech Republic, France, Ireland, Spain). The study included 2348 lymphoma cases (88% participation) and 2462 controls (81% response rate in hospital-based controls and 52% in population-based controls). Exposures were derived using an occupational exposure matrix developed by industrial hygienists and occupational experts from the research centers. Only 35 individuals (cases and controls not broken out) in the study were exposed to carbamates (glyphosate was grouped with the carbamates). No results were provided for NHL and the only OR provided for glyphosate was for B-cell lymphoma where the OR was 3.1 (0.6-17.1) based on four exposed cases and two exposed controls. No information was provided on the total number of cases for each type of lymphoma evaluated. This study has very limited power to evaluate an association between NHL and glyphosate and provides only information on B-cell lymphomas with very few exposed cases and controls. As has been done by most researchers evaluating these data, this study will receive very little weight in the evaluation of causality.

Pahwa et al. (2019) [19] pooled data from three case-control studies in the United States [8-10] and one case-control study from Canada [16] to form the North American

Pooled Project (NAPP) data set. The data set consisted of 1690 NHL cases and 5131 controls of men and women over 19 years of age. The numbers of cases and controls substantially exceeded those seen in the publications from a previous pooled analysis [11] of the three North American studies (317 more cases, 1056 more controls) due to differences in exclusion rules, most notably the inclusion of women in this pooled analysis and the imputation of age for participants with missing values for age. The number of cases from Canada was four less in the NAPP than in the original studies and there was no difference in the numbers of controls. Analyses were done for ever/never use of glyphosate, years used, days/year handled and total days handled (days/year times number of years). Missing values for years used and days/year were imputed by "simple imputation" (no methodology presented nor were the numbers of imputed exposures presented). All data from Kansas were excluded for years, days/year and total days (these data were not collected in Kansas) and all data for days/year and total days were excluded for Iowa and Minnesota (these data were not collected in Iowa and Minnesota). For days/year and total days, almost 50% of the cases and more than 40% of the controls were excluded from the analyses. Analysis was conducted using logistic regression for each of the exposure classifications first adjusting for age, sex, state/province, lymphatic or hematopoietic cancers in a first-degree relative, use of proxy respondent and use of personal protective equipment (crude analysis) and then with an additional correction for ever/never use of 2,4-D, dicamba, and/or malathion (pesticide-adjusted analysis). A similar set of analyses were done excluding the data where information is provided by proxy respondents.

The crude OR for ever/never use of glyphosate was 1.43 (1.11-1.83 95% CI) and included 1690 cases and 5131 controls. This OR was attenuated in the pesticide-adjusted analysis (1.13, 0.84-1.51). Crude analyses for years of use showed increased ORs (<3.5 years, 1.59, 1.13-2.22; >3.5 years, 1.20, 0.82-1.75) with a p-trend of 0.05 and attenuated in the pesticide-adjusted analyses (<3.5 years, 1.28, 0.88-1.84; >3.5 years, 0.94, 0.62-1.42) with no apparent trend (p-trend=0.9). An ordinal analysis using 5-year increments was significant for the crude analysis (p=0.03) and attenuated in the pesticide-adjusted analysis (p=0.3). Days/year of use showed an increased OR in the highest exposure group in the crude analysis (≤2, 1.03, 0.67-1.60; >2, 2.42, 1.48-3.96) with a significant trend (p-trend=0.002) and again attenuated in the pesticide-adjusted analysis (≤2, 0.74, 0.46-1.19; >2, 1.73, 1.02-2.94; p-trend=0.2). An ordinal analysis with increments of 5/days per year was significant in the crude analysis (p=0.02) but not in the pesticide-adjusted analysis (p=0.2). Similar patterns were seen for total days handling glyphosate (crude; ≤3.5, 1.20, 0.74-1.95; >3.5, 1.55, 0.99-2.44, p-trend=0.05, ordinal 10, p=0.02: pesticide-adjusted; ≤3.5, 0.87, 0.52-1.45; >3.5, 1.08, 0.66-1.77, p-trend=0.9, ordinal 10, p=0.08). Very similar patterns were seen for Diffuse large B-cell lymphoma (DLBCL) and small lymphocytic lymphoma (SLL) although the ORs for SLL seemed to be less attenuated when adjusted for the other 3 pesticides. Analyses excluding proxy responders showed similar patterns but with reduced statistical significance across the board except for SLL which saw very small changes in the statistical significance. Follicular lymphomas (FL) showed no association with glyphosate use. The remaining

cancers from the class of NHL were grouped together and show similar patterns as those seen for all NHL and DLBCL, but with lower statistical significance.

These findings support the existence of an association between glyphosate and NHL in general with supporting findings for DLBCL, SLL and other cancers except FL. One-third of the data were obtained from proxy-respondents so it is not surprising to see less significant findings in the sub-analysis excluding the proxy respondents. The actual ORs when excluding proxy respondents dropped for ever/never use, years of use and total days, displayed no apparent change in pattern for days/year of use. As noted by the authors, some exposure misclassification was likely to have occurred in all studies. Many of the individual studies checked for recall bias by obtaining pesticide purchase data from suppliers and comparing it to reported use and saw few differences between the measures suggesting little impact of recall bias in these studies. Thus, any exposure misclassification is likely to be non-differential pushing the results toward the null. It is unclear that imputations for exposures or for age had an impact on the results. Also, given the large reduction in sample size for the analyses done using days/year and total days handling glyphosate, it is likely the exclusions of data from Kansas, Iowa and Minnesota had an impact on these analyses relative to the other exposure metrics. The major strength of this study is the sample size.

This study will be used in the evaluation of causality.

4.3 Cohort Studies

De Roos et al. (2005)[20] reported results on the association of glyphosate and cancer incidence from the Agricultural Health Study (AHS), a prospective cohort study in Iowa and North Carolina, which included 57,311 private and commercial applicators who were licensed to apply restricted-use pesticides at the time of enrollment. Recruitment occurred between 1993 and 1997 and cohort members were matched to cancer registry files to identify cases and the National Death Index (1999) to ascertain vital status. Incident cancers were identified from the date on enrollment until 31 December, 2001, with the average follow-up time being 6.7 years. Comprehensive use data was obtained by self-administered questionnaire for 22 pesticides, ever/never use for 28 additional pesticides, and general information on work practices. Applicators were given a second self-administered questionnaire on occupational exposures and lifestyle factors. They used three exposure metrics in their analyses: a) ever personally mixed or applied pesticides containing glyphosate; b) cumulative exposure days of use of glyphosate (years of use times days per year); and c) intensity weighted cumulative exposure days (years of use times days per year times intensity of use). Persons whose first primary tumor occurred before the time of enrollment (1074) were excluded from the analysis as were those who were lost to follow-up (298), did not provide age information (7) or information on glyphosate use (1678) leaving 54,315 subjects for inclusion. There were 92 cohort members with a diagnosis of NHL during the study period of which 77.2% had

ever used glyphosate resulting in a rate ratio[8] (RR) of 1.2 (0.7-1.9) when controlling for age and an RR of 1.1 (0.7-1.9) when controlling for age, lifestyle factors, demographics and five other pesticides for which cumulative-exposure-day variables were most highly associated with glyphosate cumulative-exposure-days (2,4-D, alachlor, atrazine, metalochlor, and trifluralin) or, for chemicals with only ever/never exposure information that were most highly associated with glyphosate ever/never use (benomyl, maneb, paraquat, carbaryl and diazinon). When cumulative exposure days in exposed individuals are divided into tertiles and RRs examined using the lowest exposed tertile as the reference group, the RRs drop with values of 0.7 (0.4-1.4) and 0.9 (0.5-1.6) for tertiles 2 and 3 respectively controlling for demographic and lifestyle factors and other pesticides (30,699 subjects). When intensity-weighted exposure days are examined again using exposed tertile 1 as the reference group, the RRs drop with values of 0.6 (0.3-1.1) and 0.8 (0.5-1.4) for tertiles 2 and 3 intensity-weighted exposure days respectively controlling for demographic and lifestyle factors and other pesticides (30,699 subjects). Analyses are not shown for the evaluation of the exposed tertiles against never exposed because the authors felt that never exposed and exposed subjects differed in terms of socio-economic factors and other exposures like smoking[20].

This is a typical cohort study, but has some limitations in terms of its interpretation. The majority (75.5%) of subjects in the cohort reported having ever personally mixed or applied products containing glyphosate and was composed primarily of male, middle-aged, private applicators. For glyphosate, reliability of the answers by subjects on the use of glyphosate between the first and second questionnaire were evaluated in the AHS[120]: 82% agreement for whether they had ever mixed or applied glyphosate, 53% agreement on years mixed or applied, and 62% agreement on days per year mixed or applied and 62% agreement on decade first applied. They saw no differences in over versus under reporting between the two questionnaires suggesting this could lead to non-differential exposure bias and reduce the RRs in this study. Another weakness, noted by the authors, is that the small number of incident cases during follow-up period hindered precise effect estimates. Also, the high frequency of exposure to many pesticides (e.g. 73.8% were exposed to 2,4-D) means subjects unexposed to glyphosate were likely to be exposed to other agents that may also induce NHL, reducing the RRs. Also, as noted by the EPA's FIFRA Science Advisory Panel (SAP)[121] in their review of the EPA's issue paper on the carcinogenicity of glyphosate and as noted in a critique[122] of the European Food Safety Agency's risk assessment for glyphosate, the follow-up time in this cohort study may not be long enough to produce a sufficient sample size for evaluation of the association between NHL and glyphosate. Like other studies, this study has few exposed cases and controls, but the authors adjust their analysis for many other pesticides which could reduce ORs and increase confidence bounds limiting the ability of the study to show positive results. This study could also

[8] The rate ratio (RR) is estimated as the incidence in the exposed population divided by the incidence in the unexposed population. Incidence is calculated as the number of events in a fixed period of time divided by the person years at risk. Unlike the OR, the RR does not require the assumption of a rare disease to serve as a good estimate of the population risk ratio (PRR).

suffer from a survival bias because pesticide applicators were recruited as case participants after their exposure had begun and those with a cancer prior to enrollment were excluded.

This study will be included in the evaluation of causality.

Andreotti et al. (2017)[21] reported results on the association of glyphosate and cancer incidence from the AHS, a prospective cohort study in Iowa and North Carolina, which included 57,310 private and commercial applicators who were licensed to apply restricted-use pesticides at the time of enrollment. This study is a follow-up to the earlier report by **De Roos et al. (2005)**[20] which includes new cancers identified since the 2005 study and new information on exposure and usage. Recruitment for the AHS occurred between 1993 and 1997, and **Androetti et al. (2017)** used initial enrollment information to conduct their follow-up study. After exclusion of individuals who had a history of cancer at enrollment and those providing no information on glyphosate use, there were 54,251 cohort members available for this follow-up. Cancer incidence in this follow-up were obtained from cancer registry files in North Carolina and Iowa and vital status was identified using National Death Index and state mortality registries. Incident cancers were identified from the date of enrollment until December 31, 2013 in Iowa and until December 31, 2012 in North Carolina. In addition to the original data on glyphosate use from **De Roos et al.** (**2005**), comprehensive use data was obtained by telephone questionnaire that was administered between 1999 and 2005. Only 63% of the cohort responded to the questionnaire so the authors used a multiple imputation procedure to impute glyphosate exposure for the remaining 37% of the cohort[22] prior to 2005. They used three exposure metrics in their analyses: a) ever personally mixed or applied pesticides containing glyphosate; b) cumulative exposure days of use of glyphosate (years of use times days per year); and c) intensity weighted cumulative exposure days (years of use times days per year times intensity of use).

There were 575 cohort members with a diagnosis of non-Hodgkin lymphoma (NHL) during the study period of which 82.8% had ever used glyphosate; no rate ratio (RR) was provided for an ever-never use comparison. The authors grouped cumulative exposure days in exposed individuals into quartiles and provided RRs for each quartile compared to unexposed individuals. The RRs are below 1 but increasing with exposure with values of 0.73 (0.54-0.98), 0.80 (0.60-1.06), 0.86 (0.65-1.15) and 0.78 (0.58-1.05) for quartiles 1,2,3 and 4 respectively controlling for age, smoking, alcohol usage, family history of cancer, state and exposure to pesticides atrazine, alochlor, metolachlor, trifuralin and 2,4D. The authors also grouped intensity-weighted lifetime days in exposed individuals into quartiles and examined RRs using the unexposed group as the reference group. The RRs are again below 1 but increasing with exposure with values of 0.83 (0.59-1.18), 0.83 (0.61-1.12), 0.88 (0.65-1.19) and 0.87 (0.64-1.20) for quartiles 1,2,3 and 4 respectively. Analyses were also done using 5-, 10-, 15- and 20-year lag times. No significantly increased RRs were seen in these analyses although the general trend was toward higher RRs in the exposure groups as the lag times increased. Analyses were also presented for individual cancer classifications within the non-Hodgkin lymphoma family including B-cell NHL, chronic and small lymphocytic leukemia, diffuse large B-cell

lymphoma, marginal-zone lymphoma, follicular lymphoma, multiple myeloma, and T-cell NHL. The results were similar for the subgroupings as they were for the combined NHL with the exception of T-cell NHL where the RRs for lifetime days of exposure were 3.83 (0.84-17.49) for exposure below the median with no lag, and 2.49 (0.95-6.57) for 20-year lag and for intensity-weighted lifetime days were 4.25 (0.73-24.64) for exposure below the median with no lag, and 2.97 (1.20-7.31) for 20-year lag based on a total of 22 cases. The authors concluded there was no association between glyphosate use and NHL.

As noted for the earlier study[20], this is a typical cohort study, but has several limitations in terms of its interpretation. In **De Roos, et al. (2005)**, three-quarters (75.5%) of the subjects in the cohort reported having ever personally mixed or applied products containing glyphosate. Reliability of the answers by subjects on the use of glyphosate between the first and second questionnaire were evaluated in the AHS [120]: 82% agreement for whether they had ever mixed or applied glyphosate, 53% agreement on years mixed or applied, 62% agreement on days per year mixed or applied, and 62% agreement on decade first applied. No such comparison has been provided for this evaluation (the third time the questionnaire is applied), but it is highly likely the same lack of agreement is present. This leads to an increase in non-differential exposure misclassification and reduces the RRs in this study.

Unlike the 2017 AHS publication that compares exposure response to unexposed cohort members, **De Roos, et al. (2005)**, provided risk ratios for exposure response by comparing to the lowest exposure grouping (the exposures were given in tertiles and the exposure-response was compared to the lowest tertile) because the authors felt that never exposed and exposed subjects differed in terms of socio-economic factors and other exposures like smoking[20]. **Andreotti et al. (2017)** did not use this same reasoning, and the article does not discuss why there is a departure from this observation. Since the rate ratio is estimated as the incidence in the exposed population divided by the incidence in the unexposed population, the rate ratio against the lowest exposure would simply be calculated as the rate ratio of each exposed group divided by the rate ratio for the lowest exposed group (cancelling out the unexposed group). This would lead to rate ratios for the quartile analyses of lifetime days of q1=1, q2=1.096, q3=1.118, and q4=1.053 and for intensity q2=1, q3=1.06 and q4=1.048. Thus, unlike the previous study, this study shows increased RRs for NHL relative to the lowest exposure group.

The imputed exposures in this evaluation could also lead to non-differential exposure misclassification. This issue has been discussed before[123, 124]. **Acquavella et al. (2006)**[123] used the method for classifying exposure developed for the AHS[125] to evaluate the agreement between concentrations of glyphosate, 2,4-D and chlorpyrifos using usage data based on field observers and farmer recall. When farmer-based exposure information was used, the Spearman correlation coefficient was below 0.25 for all three compounds indicating a serious lack of agreement. **Blair et al. (2011)**[124] performed a similar analysis on 83 pesticide applicators from the AHS on 2,4-D and chlorpyrifos. They saw Spearman correlations of 0.4 for 2,4-D (n=64), 0.8 for liquid

chlorpyrifos (n=4) and 0.6 for granular chlorpyrifos (n=12). They then demonstrated that for a variety of study sensitivities and underlying RRs, there is substantial attenuation of the RR towards the null when the correlations are in the range they observed. For example, if the true relative risk is 2.0, the spearman correlation coefficient between glyphosate exposure and urinary concentration is 0.4 (close to what was seen in the study by **Acquavella et al. (2006)**, the specificity is 0.7 and the sensitivity is <0.9, the observed RR is expected to be below 1.2. They were also able to show that the misclassification is likely to be non-differential. Thus, when using the farmer's own response to calculate exposure, there is likely to be substantial attenuation to no association. Imputing answers from other farmers' responses to the 37% of the cohort that failed to respond to the questionnaire is likely to magnify the impact of non-differential exposure misclassification.

Glyphosate use in the United States has increased dramatically over the course of the AHS. Using USDA and EPA data, agricultural use in the US was 12,474, 35,720, 71,144 and 106,963 thousand kilograms in 1995, 2000, 2005 and 2010 respectively[126]. Thus, during the critical windows during which exposure histories were being obtained for the most recent questionnaire (1999-2005), agricultural use of glyphosate doubled in the U.S. and from 1999 to 2010 agricultural use tripled, mostly due to the introduction of genetically modified crops that are resistant to glyphosate. Farmers interviewed at the beginning of this time period (1999-2002) are likely to have much smaller exposures than those interviewed toward the end of this period. Using the information over this period as indicative for the entire period will clearly underestimate exposure for the entire period with the underestimation being worse for the early interviewees than for the late interviewees. They then use the information from the 63% that responded to the questionnaire to impute exposures for the remaining 37%; this imputation will compound the problem of exposure misclassification. The algorithm and methods used for the exposure imputation are provided in **Heltshe et al. (2012)**[22]. Of the 38 pesticides they evaluated, 33 had smaller values for prevalence of pesticide use from the 1999-2005 survey in the cohort members who responded as compared to the non-respondents. For glyphosate, the prevalence in respondents was 52.73% whereas for non-respondents it was 45.2%. This suggests either a systematic bias towards imputing no exposure or there is some aspect of non-response that is correlated with cohort members having less exposure during this period. If the bias is systematic, this would lead to a differential exposure misclassification potentially assigning cohort members to the unexposed group when they are really exposed.

Finally, in order to evaluate the accuracy of the imputation procedure, **Heltshe et al. (2012)**[22] withheld a subset of the data from respondents, imputed their responses and compared them using a Brier score. The Brier score is a measurement of the quality of a prediction when the predictions are probabilistic as is the case for the imputed exposures; the smaller the Brier score, the more accurate the imputed exposures. Of the 38 pesticides for which exposures were imputed, glyphosate had the worst Brier score, 0.225; this score, at best, shows a very weak degree of accuracy in the predictions.

This study will be used in the evaluation of causality.

Leon *et al.* (2019) [23] formed the AGRICOH Consortium to evaluate the relationship of 33 pesticides (including glyphosate) with NHL in a pooled analysis of three large agricultural worker cohorts. The three cohorts were the AHS (described above for Andreotti et al. 2017), the AGRGICAN cohort [24] and the CNAP cohort [25]. While they used the AHS cohort, they excluded 4619 commercial applicators (non-farmers) used in the Andreotti et al. (2017) study and included 1620 farmers with no information on frequency of exposure that were excluded from Andreotti et al. (2017). The Agriculture and Cancer (AGRICAN) cohort consists of 181,747 farm owners and farm workers (male and female) over 18 years of age who were affiliated with the French national health insurance system for farm workers for at least 3 years and who resided in one of the 11 departments in France covered by population cancer registries. They were enrolled between 2005 and 2007 and cancer and mortality were assessed up to the end of 2009. Participants completed self-administered questionnaires regarding cultivation of 13 crops and 5 animal species and on the performance of various pesticide treatment tasks. Exposure to the various pesticides was assessed using crop-exposure matrices (CEM_1) specific to France that were derived based upon what chemicals were authorized and recommended for what crops in what years. The Cancer in the Norwegian Agriculture Population (CNAP) cohort consists of 147,134 farm-holders (owners and non-owners using a farm, male and female) who had participated in at least one of five national agricultural and horticultural censuses conducted in 1969, 1974, 1979, 1985 and 1989. The census included information on crops and livestock produced, acreage, technology, pesticide expenses and pesticide spraying equipment. Exposure to the various pesticides was assessed using crop-exposure matrices (CEM_2) specific to Norway that were derived based upon what chemicals were sold and registered for use in specific years. Cancers were assessed using the Norwegian National Cancer Registry up to the end of 2011. AGRICAN had more females (44%) than the other two cohorts (16% CNAP and 3% AHS) while CNAP contributed the bulk of the person-years of follow-up with 2,396,595 person-years compared to 751,880 for AHS and 426,340 for AGRICAN. The largest crops reported differed also among the three cohorts with 70% of AGRICAN members reporting cultivating hay, meadows and grasslands, 32% in CNAP reporting potatoes and 74% of AHS reporting corn. The majority of the NHL cases were from CNAP (1498) with AHS (493) and AGRICAN (439) contributing many fewer cases.

Leon et al. (2019) reported results for ever/never use of glyphosate only and did not consider lag times or any type of exposure-response analysis. Over 80% of the participants from the AHS reported ever using glyphosate whereas less than 40% used glyphosate in the other two cohorts according to the two CEMs. Minimally- and fully-adjusted (including other pesticides) analyses were run for each cohort and then combined using random effects meta-analysis. The fully-adjusted meta hazard ratio (mHR) for glyphosate and NHL was 0.95 (0.77-1.18) and the crudely-adjusted mHR was 0.98 (0.76-1.25). Separate analyses were also done for various subtypes of NHL. For chronic lymphocytic leukemia and small lymphocytic lymphoma (CLL) the mHRs were 0.92 (0.69-1.24) fully adjusted and 1.09 (0.70-1.70) crude. For diffuse large B-cell lymphoma (DLBCL) the mHRs were 1.36 (1.00-1.85) fully adjusted and 1.12 (0.86-1.45)

crude. For follicular lymphoma (FL) the mHRs were 0.79 (0.52-1.21) fully adjusted and 0.95 (0.70-1.45) crude. For multiple myeloma and plasma-cell leukemia (MM) the mHRs were 0.87 (0.66-1.15) fully adjusted and 1.00 (0.83-1.21) crude. There was evidence of heterogeneity among the three cohorts for NHL (I^2=57%) but no heterogeneity among the cohorts for the various subtypes of NHL.

The biggest strengths of this study are the sample sizes and the cohort design. The greatest limitation of this study is that AGRICAN and CNAP are studies of crop and livestock production with no self-reported use of glyphosate exposure. Registration of glyphosate and recommendations for use on a specific crop do not guarantee it will be used on that crop which would lead to overestimation of exposure. In addition, off-label use or use for weed control around fields could have occurred which would lead to an underestimate of exposure. It is likely that this exposure misclassification is non-differential reducing statistical power and lowering mHRs towards the null (1.0). They also did not account for re-entry tasks in the questionnaires, only spraying tasks. This could also lead to exposure misclassification in the two European cohorts.

To evaluate the quality of their CEMs with respect to self-reported exposure, **Brouwer et al. (2016)** [127] applied both CEM_1 and CEM_2 (modified for US registration and recommendations) to members of the AHS cohort who completed the phase II questionnaires. Agreement between self-reported use and the assigned exposure was poor with CEM_1 showing only 65.7% agreement for glyphosate and CEM_2 showing 57.8% agreement. In a letter to the editors of the journal, **Tomenson (2017)** [128] argued that the exposure misclassification introduced by these CEMs could be differential and in either direction making the pooling project difficult to interpret and utilize. The authors disagreed [129] about the overall value of the pooling effort, but acknowledged the limitations noted by **Tomenson (2017)**.

This study will be included in the assessment of causality.

5. Consistency of Associations

There is a consistency of associations across the better epidemiology studies.

Hill (1965)[4] defines consistency as the answer "yes" to the question "Has it repeatedly been observed by different persons, in different places, circumstances and times?" For these studies, the answer is indeed yes.

If the population relative risk (PRR) for an association of glyphosate with NHL were equal to 1 (no effect), then one would expect very few statistically significant results in multiple studies and that about half of the studies would have ORs or RRs below one and half above one. As noted by both the **IARC Monograph 112 (2015)**[5] and by **Chang and Delzell (2016)**[7], when comparing studies, the most reasonable comparison is to use the most-fully-adjusted risk estimates. I will mostly limit my comments to these most-fully-adjusted risk estimates.

Consistency of the associations across several epidemiology studies is not simply a

matter of seeing how many were statistically significant and how many were not but must also address the consistency of the direction of the responses. Figure 1 shows a forest plot of all ORs and RRs for ever/never exposure from the epidemiology studies discussed previously. Each horizontal line in the forest plot shows the mean estimate of the OR/RR as a black square and the 95% confidence interval around this estimate as whiskers extending left and right from the black square.

The first obvious conclusion to be drawn from Figure 1 is that all of the mean OR/RR estimates (solid squares) for the case-control studies (blue text and lines) and the **DeRoos et al (2015)** [20]cohort study are consistently ≥1; the only exception is **Leon et al. (2019)** [23]. This implies that most of the studies are pointing in the same direction toward a positive effect. The nine studies shown in Figure 1 will be referred to as the nine core epidemiology studies. As noted above, if the true underlying risk ratio was 1 (no effect), you would expect about half of the findings to be below 1 and half to be equal to 1 or greater. Using only the results from the 8 core studies with ever/never evaluations, you can see that seven are ≥1; the probability of this happening is 0.035, strongly suggesting the studies do not agree with an underlying PRR=1 and that they consistently support a positive effect.

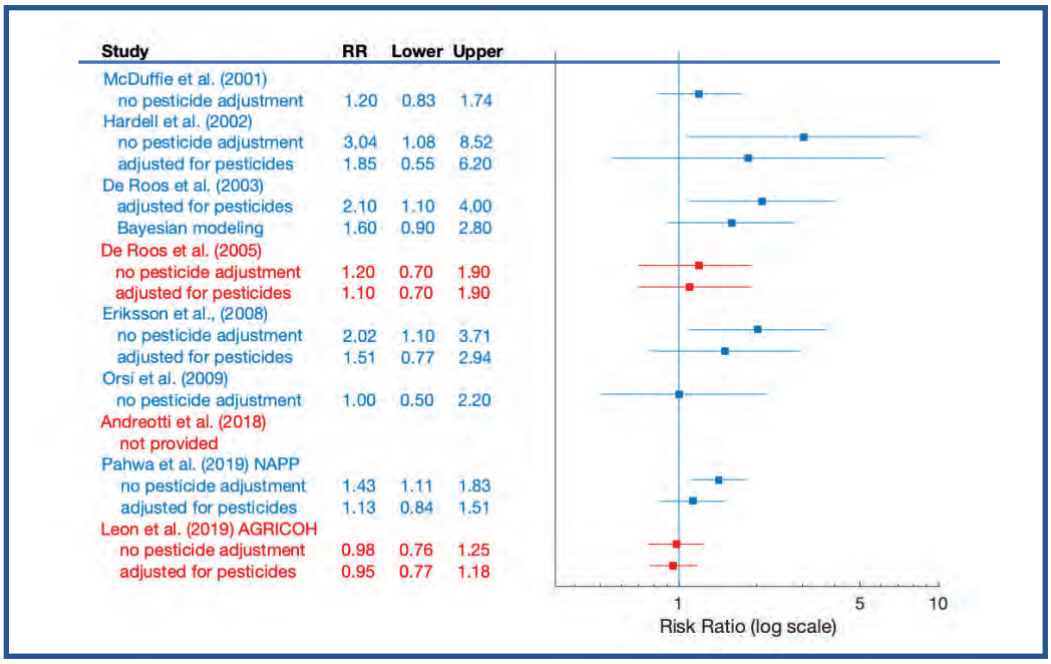
A second way in which consistency can be evaluated is to combine the individual studies using meta-analysis to obtain a combined analysis using both the ORs and the RRs (CRR) and test for heterogeneity in the studies. Four meta-analyses have been conducted for different combinations of the studies shown in Figure 1; **Chang and Delzell (2016)** [7]**, IARC (2015)** [5], **Schinasi and Leon (2014)** [26]**,** and **Zhang et al. (2019)** [27]. **Chang and Delzell (2016)** did four separate meta-analyses on the glyphosate epidemiology studies using two different methods (random-effects and fixed-effects models); their model number 2 matched exactly what was done by **IARC (2015)**. Figure 2 shows a forest plot with the principle analyses from each of these meta-analyses and the underlying individual evaluations from the individual studies. The analyses by **IARC (2015)** [5] and **Schinasi and Leon (2014)** [26] are effectively covered by the analyses of **Chang and Delzell (2016),** so I will focus on only **Chang and Delzell (2016)** [7] and **Zhang et al. (2019)** [27].

In their first analysis (Model 1, see Figure 2)[9], they combined the most-fully-adjusted risk estimates from six of the core studies to yield a CRR of 1.27 (1.01-1.59) for both random-effects and fixed-effects models supporting an association between NHL and glyphosate exposure in these studies. In a second analysis (Model 2), they replace the results of the Bayesian analysis in **De Roos et al. (2003)** with the results of the logistic regression analysis and get the same CRR of 1.30 (1.03-1.64) for both random-effects and fixed-effects models. In a third analysis (Model 3), they replace from Model 1 the **McDuffie et al. (2001)** results in with a combined meta-analytic result they derived from analyses by **Hohenadel et al. (2011)** (this study reanalyzed the same data as **McDuffie et al. (2001)**, splitting results between asthmatics and non-asthmatics) resulting in a CRR of

[9] **Chang and Delzell (2016)** provided only one significant digit to the right of the decimal point in their confidence bounds; the EPA SAP (2017) re-calculated models 1-4 of **Chang and Delzell (2016)** to provide two significant digits – these are presented here.

1.32 (1.00-1.73) for both random-effects and fixed-effects models. Finally, in a fourth analysis (Model 4), they use Model 3 but replaced the Bayesian analysis in **De Roos et al. (2003)** with the logistic regression analysis yielding a CRR of 1.37 (1.04-1.82) for both random-effects and fixed-effects models. In essence, all of the different meta-analyses resu

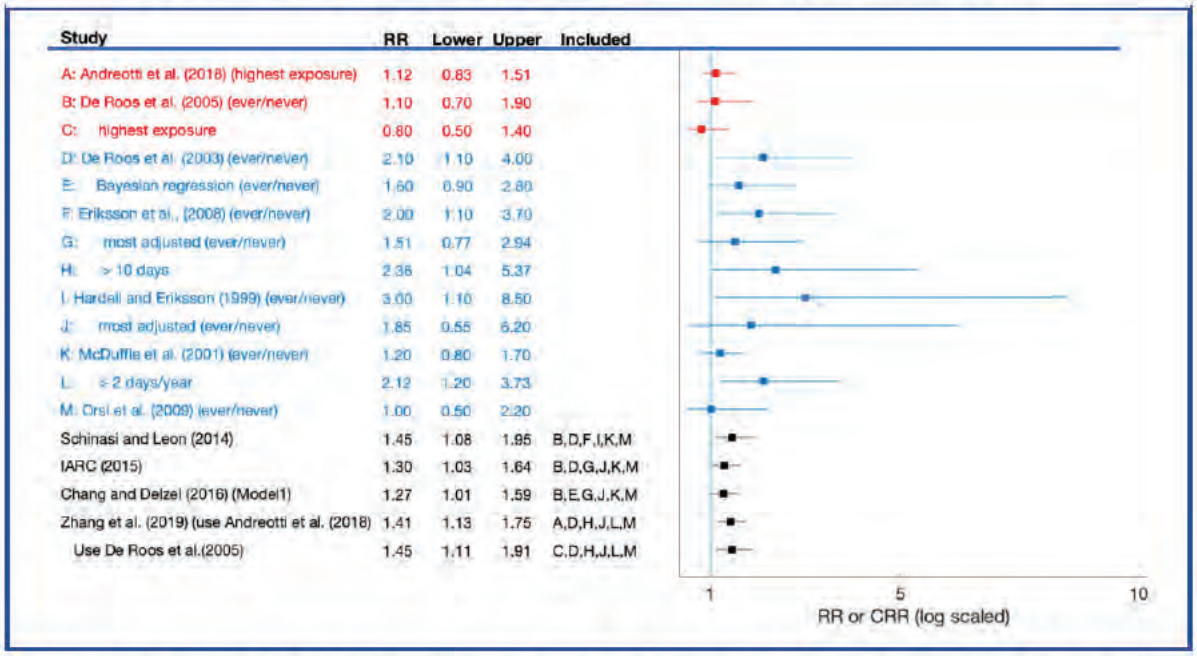
Figure 1: Odds Ratios and Rate Ratios for ever/never exposure from selected epidemiology studies.



("RR" refers to the OR, RR or mHR from the study, "Lower" refers to the 95% confidence lower bound and "Upper" to the 95% confidence upper bound. Case-control studies are shown in blue and cohort studies in red.)

As stated above, another way to evaluate consistency in the epidemiological data would be to evaluate the heterogeneity in the studies. Heterogeneity may be due to differences in participants, outcomes, exposure metrics, methods for questioning study subjects, sex of the subjects, etc. **Chang and Delzell (2016)** formally tested for heterogeneity of the responses from the six core studies using Cochran's Q statistic and the I^2 statistic[130]. For models 1 to 4, the p-values from Cochran's Q test are 0.84, 0.59, 0.85, and 0.63 respectively (typically you reject the concept of homogenous studies in favor of heterogeneous studies if $p<0.10$). The I^2 statistic for all four models are 0.0% (values for I^2 can range from 0-100% with concern for heterogeneity above 50%). The fact that the fixed-effects models and random-effects models gave the same results also supports a lack of heterogeneity in the data. There is no indication of heterogeneity in these six core studies. Lack of heterogeneity supports the interpretation that the meta-analyses as showing a positive association and strong consistency of the findings across the six core studies.

Figure 2: Odds Ratios and Rate Ratios for ever/never exposure from selected epidemiology studies and the meta-analyses done on these studies.



("RR" refers to the OR or RR from the study, "Lower" refers to the 95% confidence lower bound and "Upper" to the 95% confidence upper bound. Case-control studies are shown in blue, cohort studies in red and meta-analyses in black.)

Chang and Delzell (2016) also evaluated the association between subtypes of NHL and glyphosate exposure where possible. For B-cell lymphomas, they combined the results of **Eriksson et al. (2008)**[15]with those of **Cocco et al. (2013)**[18] and saw a CRR (random-effects and fixed-effects) of 2.0 (1.1-3.6) with an I^2 of 0 and a Cochran's Q test p-value of 0.58. For diffuse large B-cell lymphomas, they combined the results of **Eriksson et al. (2008**)[15] with those of **Orsi et al. (2009)**[17] and saw a CRR (random-effects and fixed-effects) of 1.1 (0.5-2.3) with an I^2 of 0 and a Cochran's Q test p-value of 0.79. For combined chronic lymphocytic leukemia and small lymphocytic lymphoma, they combined the results of **Eriksson et al. (2008)**[15] with those of **Orsi et al. (2009)**[17] and saw a CRR using the random-effects model of 1.3 (0.2-10) and for the fixed effects model 1.9 (0.9-4.0) with an I^2 of 83.7% and a Cochran's Q test p-value of 0.01. For follicular lymphomas, they combined the results of **Eriksson et al. (2008)**[15]with those of **Orsi et al. (2009)**[17] and saw a CRR (random-effects and fixed-effects) of 1.7 (0.7-3.9) with an I^2 of 0 and a Cochran's Q test p-value of 0.73. And finally, for HCL, they combined the results of **Nordstrom et al. (1998)**[12] with those of **Orsi et al. (2009)**[17] and saw a CRR (random-effects and fixed-effects) of 2.5 (0.9-7.3) with an I^2 of 0 and a Cochran's Q test p-value of 0.63. These subtype analyses are based upon small numbers of cases and only two studies making them unreliable, when considered individually, to address the question of consistency in the data. However, when they are combined with the results for the meta-analyses of the core studies of NHL, these studies add support to the conclusion that these data are consistent.

Chang and Delzell (2016) also performed a sensitivity analysis by only doing meta-

analyses on studies with similar characteristics. Using only the five case-control studies, the CRR was 1.3 (1.0-1.7). Breaking them into the type of control used, there were four studies using population controls with a CRR of 1.4 (1.0-1.8). There were four studies with males only with a CRR of 1.3 (1.0-1.7) and two studies with males and females with a CRR of 1.2 (0.8-1.8). Three studies were done in North America with a CRR of 1.2 (1.0-1.6), three in Europe with a CRR of 1.3 (0.8-2.1); two of the three studies were in Sweden with a CRR of 1.6 (0.9-2.8). All of the resulting meta CRRs were the same for the fixed-effects model and the random-effects model. This sensitivity analysis shows that the results do not differ significantly from the main CRR for the six core studies combined adding support to the findings being consistent across the different studies.

Zhang et al. (2019) [27] conducted a meta-analysis that includes the study by **Andreotti et al** (2018) [21] using the highest exposure groups, when available, from each study (see Figure 2). They saw an increased CRR of 1.41 (1.13-1.75) for the fixed effects model and 1.52 (1.12-2.16) for the random effects model. When they used the highest exposures from the **DeRoos et al (2005)** study rather than **Andreotti et al. (2018)**, the CRRs were 1.45 (1.11-1.91) for the fixed effects model and 1.52 (1.00-2.31) for the random effects model. Using the longest duration rather than the highest exposure yielded similar results. Excluding the AHS study and only including the highest exposures from the case-control studies yielded CRRs of 1.84 (1.33-2.55) for the fixed effects model and 1.86 (1.39-2.48) for the random effects model. They also did a variety of sensitivity analyses to determine if the results were sensitive to the inclusion of certain studies, different exposure measures, different geographical regions, etc. In all cases, the resulting CRR was greater than 1 and in nearly all cases had a 95% lower confidence bound above 1.

No published meta-analysis are available including the **Leon et al. (2019)** and **Pahwa et al. (2019)** studies. Because the **Pahwa et al (2019)** study is positive for a linkage between NHL and glyphosate exposure, it is unlikely to substantively change the meta-analyses seen above. It is unclear what effect the **Leon et al. (2019)** study would have on a meta-analysis against the other studies. However, given its unique use of CEMs to estimate exposures and the problems associated with the **Andreotti et al. (2018)** study discussed previously, a meta-analysis using these data should carry less weight in the overall evaluation.

In case-control studies, selection bias arises when the reasons cases and controls choose to participate in the study could lead to systematic biases that might result in a positive or negative finding independent of the exposure being studied. For example, if cases with exposure are more likely to participate than controls with exposure, the result would be higher OR values; however, this difference has to be differential and not simply a difference in participation rates. It is possible that in a few of these studies, the method by which controls were selected could contribute to selection bias that might lead to increased ORs. However, given the diverse types of cases and controls used in the five core case-control studies, this is unlikely to explain the consistent findings seen from these studies. It is also possible that the lack of complete data on cases versus controls could result in selection bias if the reasons for not completing the

questionnaire/interview are different between cases and controls and relates to exposure. There is no indication of this type of selection bias in these reports, and this is unlikely to explain the consistency seen in these data.

Exposure misclassification can lead to increases or decreases in the OR or RR values seen in both case-control and cohort studies. For example, in case-control studies, if cases are more likely to say they were exposed to glyphosate than controls, this would inflate the OR values; this is one type of recall bias. This type of bias is less likely in cohort studies. In all of the core studies, this issue was discussed by the authors. In every case, they concluded there was bound to be some exposure misclassification, but that it was most likely non-differential, meaning that the misclassification was random; this would likely reduce the OR/RRs seen in the studies rather than increase them. However, for the **Andreotti et al (2018)** study, it is possible there is some differential exposure misclassification as discussed above.

Confounding occurs when there is an exposure or some other factor that is tightly associated with both glyphosate exposure and NHL diagnosis that, if controlled for, could explain the results. The most likely source of confounding in these studies would be exposures to other pesticides. Seven [11, 14, 15, 19-21, 23] of the core studies controlled for exposure to other pesticides. Another concern for confounding would be if the cases had immune deficiencies that could be linked to NHL; in all of the case-control studies, such cases were excluded. Finally, other agricultural exposures (e.g. animals, other chemicals, infectious agents) could be correlated with glyphosate exposure and may be linked to NHL; none of the studies controlled for these factors. However, not all exposed cases were farmers; if confounding via other agricultural exposures is occurring, it is not possible to determine the magnitude or direction of such an effect from these data.

In conclusion, we have seven epidemiology studies done on two different continents by different research groups using different designs, questionnaires and study populations that are very consistent with no obvious bias or confounding that would explain the results. There are two additional studies, **Andreotti et al. (2018)** and **Leon et al. (2019)** that are not independent, have problems with exposure misclassification and differ from the other seven in response. **There is a consistency of associations across the better epidemiology studies.**

6. Strength of the Association seen in Human Epidemiological Studies

There is a strong association across the better epidemiology studies.

To explain strength of association, **Hill (1965)** gives the classic example of John Snow and the cholera epidemic of 1855 where the risk ratio of dying if you drank water from the Southwark and Vauxhall Company (polluted by sewage) compared to drinking from the Lambeth Company water (sewage free) was 14. Yet, for the six core studies, the OR/RR for ever/never exposure ranges from 1.0 to 1.85 for the most-fully-adjusted risk estimates and to 2.1 if you include the fully adjusted risk estimate from De Roos et al.

(2003)[20] using logistic regression. These are moderate OR/RR estimates making it conceivable they are individually due to either chance or bias. Thus, with the exception of the logistic regression analysis in **De Roos et al. (2003)** [20], none of the core studies demonstrate large, precise risks as envisioned by **Hill (2016) [4]**. However, **Hill (1965)** was not expressing himself in statistical terms where the significance of an association is dependent upon the precision of the observations. If the statistical variation around an OR/RR estimate is large relative to the estimate itself, the estimate is not very precise and generally would not be statistically significant. The result from the study by **Hardell et al. (2002)** shown in Figure 1 is an example of an estimate with very large statistical variation. On the other hand, a very small (in value), precise OR or RR estimate could be statistically significant and prove important in deciding causation. The meta-analyses shown in Figure 2 all demonstrate estimates of CRR that are significantly different from 1 rejecting the concept that the overall association is due to chance. The statistically significant estimate of the CRR for B-cell lymphomas in the meta-analysis also support this finding as does the NAPP pooled analyses demonstrating consistent positive findings.

In summary, we have seven of the nine core epidemiology studies that all show approximately the same, modest increase in OR/RR that, when combined, demonstrate a significant strength of association. **There is a strong association across the better epidemiology studies**

7. Biological Plausibility

The evidence for biological plausibility is overwhelming. Glyphosate clearly causes multiple cancers in mice, two cancers in the hematopoietic system similar to what is seen in humans, causes cancer in rats, is genotoxic and induces oxidative stress. The findings are clear for both glyphosate alone and for glyphosate formulations. There is strong support for biological plausibility in support of a causal association of glyphosate and glyphosate formulations with NHL.

The range of data one can use to determine biological plausibility is quite diverse and can be exceptionally complicated. For simplicity, it can be divided into the types of assays that can be used in this evaluation: animal cancer bioassays, toxicokinetic studies, studies from accidental exposures in humans, and studies of specific biological mechanisms in animals or cells derived from humans or animals. Animal cancer bioassays are intended to test whether glyphosate can cause cancers in mammals, thus supporting the concept that the chemical could cause cancer in humans. Toxicokinetic studies provide insight into the degree to which glyphosate is absorbed by humans, distributed to various organs in the body, what happens to the chemical once it is in the body (metabolism), and, finally, how it is eliminated from the body. Studies from accidental exposures in humans can provide some information on the effects of glyphosate through changes in the chemistry and cellular structure of human blood. Studies of biological mechanisms are generally addressing what effects the chemical may have on human and animal cells under controlled, laboratory conditions. Some of the studies in this section were done with technical grade (virtually pure) glyphosate

and some with the glyphosate formulations that humans encounter in occupational and environmental settings. I will summarize the literature in each of these areas and offer an opinion to their support of biological plausibility of NHL in humans.

7.1 Animal Cancer Bioassays

7.1.1 Basic Introduction

NOTE: Some of the text and analyses in this section are included in **Portier (2020)** [131]. Some text and tables from that publication are included verbatim in this report without attribution.

Typical animal cancer bioassays will expose animals (generally rats or mice) to a chemical for a substantial proportion of the animal's life (generally 2 years, sometimes 18 months) then kill the animal and examine its organs and tissues for tumors. There are guidelines on how to conduct and analyze these studies. Typically, chemical registrants conduct cancer bioassays for pesticide approval pursuant to guidelines developed under the guidance of the Organization for Economic Cooperation and Development (OECD[28]). Other groups[1, 3, 6] provide guidance on how to analyze these studies based upon methodology papers from the published literature. These studies are conducted in a way that controls for everything in the animal's environment (e.g., food type, water quality, how often the animals are handled) leaving only the exposure to explain differences in tumor formation between control and exposed animals. Even then, non-cancer endpoints can also be modified by the chemical and these may have an impact on tumor rates in the animals (e.g., survival, death from some other toxic effect of the chemical); these must be accounted for when reaching conclusions from the study.

Studies generally use four groups of animals, one group receiving no exposure (control) and the remaining three groups are test animals, with each group receiving different dose exposures to the chemical[29]. Doses generally above human experience are used in animal carcinogenicity studies because only relatively small numbers of animals are being used to evaluate risk for a large human population and because even the best known human carcinogens do not cause cancer in large fractions (say 20%) of the human population. The basic underlying premise of this design consideration is that, as the dose increases, so does the risk of getting a tumor. By exposing animals to the highest dose possible, you increase the ability of the study to identify a risk if one is present. However, one must be careful not to use a dose that is so high it will cause cancers by processes that would never work at lower doses. To avoid this, studies are designed around a maximum tolerated dose (MTD). This dose is generally determined based upon a subchronic study (90 days) in the same animals and is usually the maximum dose that can be tolerated by the animals without any signs of significant toxicity in the exposed animals (e.g., weight loss, tissue damage). The OECD and EPA provide guidelines[3, 28] on how to choose this top dose. These guidelines are in general agreement with the scientific literature[29].

The guidelines also address the methods by which the data should be analyzed. For example, the EPA guidelines[54] state that:

"*A trend test such as the Cochran-Armitage test (Snedecor and Cochran, 1967) asks whether the results in all dose groups together increase as dose increases. A pairwise comparison test such as the Fisher exact test (Fisher, 1950) asks whether an incidence in one dose group is increased over that of the control group. By convention, for both tests a statistically significant comparison is one for which p is less than 0.05 that the increased incidence is due to chance. Significance in either kind of test is sufficient to reject the hypothesis that chance accounts for the result.*"

In fact, most guidelines and peer-reviewed publications come to the same conclusion[6, 28, 29, 132] on what tests to use, as did EPA's FIFRA Scientific Advisory Panel (SAP) in their review of the EPA's issue paper of the carcinogenicity of glyphosate[121]. The US National Toxicology Program (NTP) uses both a trend test[88, 89, 133] and Fisher's exact test for analyzing carcinogenicity data. Unless otherwise noted in this document, all p-values presented in this section on animal cancer studies were recalculated on my computer and are the exact one-sided p-values for the Fisher test (p_{Fisher}) and/or the Cochran-Armitage linear trend test (p_{Trend}) where appropriate.

Individual tumor counts for the individual studies are reanalyzed using the exact form of the Cochran-Armitage (C-A) linear trend test in proportions [59]. Reanalyses are conducted on all primary tumors where there are at least 3 tumors in all of the animals in a sex/species/strain combination (regardless of dosing). In addition, any tumor where a positive finding (p≤0.05, one-sided C-A trend test) is seen in at least one study is also evaluated, regardless of number of animals with the tumor, in all studies of the same sex/species/strain. When adenomas and carcinomas are seen in the same tissue, a combined analysis of adenomas and carcinomas is also conducted. The minimum of three tumors is used since the exact version of the C-A test cannot detect tumors in studies of this size with less than at least 3 tumors. Pairwise comparisons between individual exposed groups and control are conducted using Fisher's exact test [59] and are provided for comparison with other reviews.

The C-A trend test belongs to the general class of logistic regression models [59]. To evaluate the consistency of a tumor finding across multiple studies using the same sex-species-strain combinations, logistic regression with individual background responses and dose trends are fit to the pooled data using maximum likelihood estimation. In mathematical terms, the regression model being used is:

$$p = \frac{e^{\alpha_i + \beta \cdot dose}}{1 + e^{\alpha_i + \beta \cdot dose}} \quad (1)$$

where *p* is the probability of having a tumor, α_i is a parameter associated with the background tumor response (dose=0) for study *i* and β is a parameter associated with a change in the tumor response per unit dose (slope). A common positive trend is seen in the pooled analysis when the null hypothesis that the slope is 0 (H_0: β =0) is rejected (statistical p-value ≤0.05 using a likelihood-ratio test) in favor of the alternative that the slope is greater than 0 (H_A: β >0). The heterogeneity of slopes (all studies have different slopes vs all studies have a common slope) is tested using the model:

$$p = \frac{e^{\alpha_i + \beta_i \cdot dose}}{1 + e^{\alpha_i + \beta_i \cdot dose}} \quad (2)$$

where *p* and α_i are as in equation (1) and β_i is a parameter associated with the slope for study *i*. Heterogeniety is seen in the pooled analysis when the null hypothesis that the slopes are equal (H_0: $\beta_1=\beta_2=\beta_3=\ldots$) is rejected (statistical p-value ≤0.05 using a likelihood-ratio test) in favor of the alternative that at least one of the slopes is different.

To avoid doing large numbers of tests and over-analyzing the data, my comments will generally rely upon the use of the trend test with the results from Fisher's exact test serving as a descriptive discussion of the findings. This is in agreement with SAP comments[121] and is generally accepted in the evaluation of animal cancer studies.

Even with the high doses used in these studies, it is sometimes necessary to use "historical controls" to evaluate a given response, especially in cases where a tumor is rare (<1% of the animals get the cancer without chemical exposure). Historical controls are generally the historical collection of tumor responses from untreated control groups from studies in the same laboratory within two to three years of the study being evaluated [1, 6, 28, 134, 135]. Evaluation of the data using the historical controls should be done rigorously to correctly evaluate the responses seen in a given study. Where a valid historical control dataset was available, I used Tarone's trend test [60] for inclusion of the historical control data; this is labeled p_{Hist}. In all cases, the guidelines and literature support the use of the control in the current study as the most appropriate control group to use unless there is a specific need to address historical responses. Many guidelines[1, 3, 6, 136] suggest historical controls be used for evaluating rare tumors and findings in assays that appear to be unusual. It is explicitly noted that significant increases in tumors over what is seen in the concurrent control should not be rejected simply because the tumors are in the range of the historical controls[6]. Nor is it recommended to reject significant increases in tumor responses because the control response is on the low end of the historical range. Animals are randomly assigned to control and exposure groups and any low response in controls is likely to also reflect similar response patterns in treated animals. This is in agreement with SAP comments[121] on the EPA issue paper on glyphosate[54] and with all guidelines for analyzing animal carcinogenicity data.

There are 14 animal carcinogenicity studies in rats [30-43] and nine in mice [44-52]. Only two studies [33, 39] appear in the peer-reviewed literature; the remaining studies are partially available through several sources. For seven of the rat studies [30, 31, 34, 36, 40-42] and five mouse studies [44, 46, 47, 50, 51], confidential proprietary technical reports from the performing laboratory are available following the findings of court case T-329/17 in Europe[10] [53]. For the remaining studies, data was obtained from the EPA review of glyphosate [54], the European Food Safety Authority review of glyphosate [55, 56], the Joint Meeting on Pesticide Residues report on glyphosate [57] and

[10] See http://curia.europa.eu/juris/documents.jsf?num=T-329/17

supplemental material from a review of the carcinogenicity of glyphosate by a panel of scientists on behalf of Monsanto [58]. Only 13 of these studies were of sufficient quality or provided sufficient detail to be included in a reanalysis of the data. These are shown in Table 1.

Many additional endpoints, other than cancer incidence and related toxicities, were evaluated in these studies; I will only provide comments on the tumor incidence data and related data where relevant to the cancer findings.

To summarize the strength-of-evidence for each tumor, four categories are used. Clear evidence (CE) is indicated when the data demonstrate a causal linkage between glyphosate and the tumor based upon the reanalysis in this review and the available peer-reviewed literature. Some evidence (SE) is indicated when the data demonstrate a linkage between glyphosate and the tumor based upon the reanalysis in this review and the available peer-reviewed literature but chance, although unlikely, cannot be ruled out. Equivocal evidence (EE) also indicates the data demonstrate a linkage between glyphosate and the tumor based upon the reanalysis in this review and the available peer-reviewed literature, but chance is as likely an explanation for the association as is glyphosate. No evidence (NE) indicates any linkage between glyphosate and the tumor based upon the reanalysis in this review is almost certainly due to chance. The factors used to put tumors into these categories include the analyses of the individual studies, the consistency of the data across studies (the pooled analyses), the analyses using historical control data, the analyses of the non-neoplastic lesions, the mechanistic evidence and the associated scientific literature.

Table 1: Long-term chronic dietary exposure toxicity and carcinogenicity studies of glyphosate analyzed in this evaluation.

Study Reference	Duration (months)	Strain		Dietary exposure dose levels (mg/kg/day)	Animals per Group	Purity (%)
		Mouse	Rat			
A: Lankas (1981) [137]	26		SD[2]	M: 0, 3.05, 10.3, 31.49 F: 0, 3.37, 11.22, 34.02	50	98.7
B: Stout and Ruecker (1990) [40]	24		SD[2]	M: 89, 362, 940 F: 0, 113, 457, 1183	50	98.7
C: Atkinson (1993) [30]	24		SD[2]	M: 0, 11, 112, 320, 1147 F: 0, 12, 109, 347, 1134	50	98.9
D: Enemoto (1997) [34]	24		SD[2]	M: 0, 104, 354, 1127 F: 0, 115, 393, 1247	50	95.7
E: Suresh (1996) [41]	24		W[3]	M: 0, 6.3, 59.4, 595.2 F: 0, 8.6, 88.5, 886	50	96.8
F: Brammer (2001) [31]	24		W[3]	M: 0, 121, 361, 1214 F: 0, 145, 437, 1498	53	97.6
G: Wood et al. (2009) [42]	24		W[3]	M: 0, 165, 838.1, 4348 F: 0, 153.2, 786.8, 4116	51	94.7-97.6
H: Knezevich and Hogan (1983) [46]	24	CD-1		M: 0, 157, 814, 4841 F: 0, 190, 955, 5874	50	99.8
I: Atkinson et al. (1993) [138]	24	CD-1		M: 0, 98, 297, 988 F: 0, 102, 298, 1000	50	>97.0
J: Sugimoto (1997) [50]	18	CD-1		M: 0, 165, 838.1, 4348 F: 0, 153.2, 786.8, 4116	50	94.6-95.7
K: Wood et al. (2009) [51]	18	CD-1		M: 0, 71.4, 234.2, 810 F: 0, 97.9, 299.5, 1081.2	51	95.7
L: Takahashi (1999a) [52]	18	CD-1		M: 0, 167.6, 685, 7470 F: 0, 93.2, 909, 8690	50	97.5
M: Kumar (2001) [47]	18	S-A[1]		M: 0, 85.5, 285.2, 1077.4 F: 0, 104.5, 348.6, 1381.9	50	>95.0

7.1.2 Analysis of Individual Rat Studies

Reyna and Gordon (1974)[38] exposed Albino rats (probably Sprague-Dawley) to ammonium salt of glyphosate (13.85% purity) in a two-year chronic feeding study. Only EPA[54] reported on this study and provided no details other than to report there were approximately 70 animals per group and there was insufficient reporting on the histopathology findings. Because of inconsistencies in the data from this study, EPA concluded it was invalid [139]. Insufficient detail is available on this study.

This study is inadequate for use in deciding on causality.

Burnett et al. (1979)[32] exposed male and female albino rats to an aqueous monosodium salt solution of glyphosate by oral intubation (purity not given). There were 90 animals per group and doses were 0, 3, 10 and 30 mg/kg/day for 24 months.

EPA[54] reported that no histopathological alterations were observed; no additional information was available on this study. This study had severely reduced sensitivity to observe any cancer findings because the highest dose used in this study is very low compared to the MTDs in the other rat studies. EPA initially reported this as a glyphosate study [54] but later removed it because it was a study of a contaminant of glyphosate [140]. This study does not contribute to the evaluation of cancer causation in laboratory animals and will be excluded from any further discussion.

Lankas et al. (1981) [36] exposed groups of 50 male and 50 female Sprague-Dawley rats to glyphosate (98.7% purity) in feed (see Table 2 for doses) for 26 months. This study is not in concordance with OECD guidelines (they were not available at the time of this study), but as noted by EFSA [55], it was in general accordance with the 1981 OECD guidelines. Information on this study was available from EPA[54], EFSA[55], Greim et al.[58], the original study report from Bio/dynamics Inc [36].

There were no survival differences in this study and there was no indication that the highest dose used exceeded the maximum-tolerated dose.

Table 2 shows the statistically significant trend in testicular interstitial cell tumors that was observed (p_{Trend}=0.009). Historical controls were provided in the study report for five studies with response rates of 4/116, 5/75, 4/113, 6/113 and 5/118 for a mean response of 4.5% (24/535). **Lankas et al. (1981)** argued that the tumor rates at sacrifice were not statistically significant from control suggesting this finding is not related to glyphosate. However, by reducing the numbers of animals to only those at terminal sacrifice, the power to find an effect was significantly reduced. Also, if the tumor increases the animal's chances of dying, then some animals with tumors will die early, which could bias results only seen at terminal sacrifice. This type of analysis is simply never done; it appears to have been developed for this case to dismiss the effects seen in the study. **Lankas et al. (1981)** also suggested the control response was low compared to the historical rates, but the concurrent control is always the best control group to use unless it is clearly flawed[1, 3, 28]; in this case, there was no apparent problem with the controls because the probability of seeing 0/50 if the true background response is 4.5% is about 10% and this control group is not significantly different than the historical controls. Using the historical control yields p_{Hist}=0.013, showing that this result is significant, even when comparing it to the historical control dataset. **EFSA**[55] examined rates for interstitial cell hyperplasia (a potential precursor for the interstitial cell tumors) and saw no dose-response trend. However, these were very low rates would suggest that the tumors arising in the 10 animals that did get interstitial cell tumors are independent of a mechanism involving interstitial cell hyperplasia. The tumor response for interstitial cell tumors was not monotonic (tumor rates increasing as dose increases), but was still within statistical variation. The EPA SAP agrees, concluding that "requiring visual confirmation of a monotonic trend in scatter plots of data … is known to be a poor way of assessing trend"[121].

An increase in Thyroid C-cell carcinomas (Table 2) was observed in female rats (p_{Trend}=0.003) but combining adenomas and carcinomas was only marginally significant

(p_{Trend}=0.072). Independent pathologists brought in by Monsanto argued these tumors were not treatment related. The authors provided historical control data for both carcinomas and carcinomas combined with adenomas from nine control groups with responses of 0/59, 1/59, 0/59, 0/58, 3/57, 1/55, 0/58, 0/55, and 0/53 for carcinomas and 10/58, 6/59, 5/59, 6/58, 6/57, 5/55, 2/58, 0/55, and 1/53 for the combined tumors. The significance of both results was stronger using the historical control data with p_{Hist}<0.001 for carcinomas and p_{hist}=0.037 for the combined.

This study also had a statistically significant increase in pancreatic islet cell tumors in the lowest dose (p_{Fisher}=0.028) in males (Table 2), but not any of the other doses; the trend test was not significant (p_{Trend}=0.316). There was a marginally significant increase in liver carcinomas in males (p_{Trend}=0.062).

The highest dose used in this study in Sprague-Dawley rats is far below the MTD. Even though EFSA [55] noted that this study was in general accordance with the 1981 OECD guidelines, they dismissed it for not meeting current guidelines due to the low-doses used. EPA[54] also excluded this study from consideration but included it in the revised risk assessment [140]. However, the study saw an increase in testicular tumors in males and Thyroid C-cell carcinomas in females that should be carefully evaluated in determining causality. Also, this is the study with the longest exposure (26 months) and provides unique information to the overall evaluation.

Additional tumors seen to have significant increases in other studies using Sprague-Dawley Rats are also included in Table 2.

In conclusion, this study shows positive result for testes interstitial cell tumors in male Sprague-Dawley rats and a positive response for thyroid c-cell carcinomas in female Sprague-Dawley rats and will be included in the overall evaluation of causation.

Table 2: Tumors of interest in male and female Sprague-Dawley rats the 26-month feeding study of Lankas (1981)

Tumor	Doses (mg/kg/day) or Tumor Incidence[1]				Trend Test p-value
Males	0	3.05	10.30	31.49	
Testicular Interstitial Cell Tumors	0/50	3/50	1/50	6/50*	0.009 (0.013)[2]
Pancreas Islet Cell Adenomas	0/50	5/49*	2/50	2/50	0.512
Pancreas Islet Cell Carcinomas	0/50	0/49	0/50	1/50	0.251
Pancreas Islet Cell Adenomas or Carcinomas	0/50	5/50*	2/50	3/50	0.316
Thyroid C-Cell Adenomas	5/47	1/49	0/49	2/49	0.743
Thyroid C-Cell Carcinomas	0/47	0/49	1/49	0/49	0.505
Thyroid C-Cell Adenomas and Carcinomas	5/47	1/49	1/49	2/49	0.748
Thyroid Follicular-Cell Adenomas	1/47	2/49	4/49	4/49	0.122
Thyroid Follicular-Cell Carcinomas	0/47	0/49	0/49	0/49	---
Thyroid Follicular-Cell Adenomas and Carcinomas	1/47	2/49	4/49	4/49	0.122
Liver Neoplastic Nodules	3/50	3/50	1/50	3/50	0.471
Liver Carcinomas	0/50	0/50	1/50	2/50	0.062
Liver Nodules and Carcinomas	3/50	3/50	2/50	5/50	0.173
Kidney Adenomas	1/50	1/50	0/50	0/50	0.938
Skin Keratoacanthomas	0/49	0/48	0/49	0/49	---
Skin Basal Cell Tumors	0/49	0/48	0/49	1/49	0.251
Females	0	3.37	11.22	34.02	
Thyroid C-Cell Adenomas	5/47	3/49	6/50	3/47	0.679
Thyroid C-Cell Carcinomas	1/47	0/49	2/50	6/47	0.003 (<0.001)[2]
Thyroid C-Cell Adenomas and Carcinomas	6/47	3/49	8/50	9/47	0.072 (0.037)[2]
Adrenal Cortical Adenomas	5/50	10/50	6/50	4/49	0.851
Adrenal Cortical Carcinomas	1/50	0/50	1/50	1/49	0.386
Adrenal Cortical Adenomas and Carcinomas	3/50	4/50	2/50	2/49	0.801

This is publication [137]. 1 – Doses are given in the rows marked "Males" and "Females", tumor counts appear on the rows with the individual tumors; 2 – p_{hist} from Tarone's test; * $0.01<p\leq0.05$ for Fisher's Exact Test; ** $p\leq0.01$ for Fisher's Exact Test

Stout and Ruecker (1990)[40] exposed groups of 50 male and 50 female Sprague-Dawley rats to glyphosate (98.7% purity) in feed (see Table 3 for doses) for 24 months. This study was done under OECD guidelines.

There were no survival differences in this study and there was no indication that the highest dose used exceeded the maximum-tolerated dose.

Pancreatic islet cell tumors were increased in all dose groups relative to the controls in male rats and statistically significant for the lowest (p_{Fisher}=0.015) and highest (p_{Fisher}=0.032) dose groups (Table 3). There was no significant trend in these data (p_{trend}=0.206). Historical control data for this tumor in this laboratory was reported as 23/432 or 5.3% [141] and Tarone's test using historical control rate was significant (p_{hist}<0.001). The lack of a trend is driven by the up and down nature of the response. Assuming the historical rate of 5.3% is correct, the chances of seeing eight or more tumors in 47 animals is 0.003. Similarly, for the mid- and high-doses, this probability is 0.124 and 0.014, respectively. Females did not show an increase in this tumor. The authors provided a table with the combined results for pancreatic islet-cell adenomas and carcinomas from this study with the tumor counts from the **Lankas et al. (1981)** [36] study arguing the results do not show a dose-related increase. Animals studied for 26 months versus 24 months can have very different responses to the same chemical and very different control incidence.

In male rats, there was a statistically significant trend (p_{Trend}=0.015) after removal of interim-sacrificed animals for hepatocellular adenomas but a significant increase for adenomas and carcinomas combined (p_{Trend}= 0.05, Table 3) and not in females (not shown). Liver carcinomas are generally also provided in a separate analysis, but these data were not provided by the authors (the data would suggest the hepatocellular carcinomas would have a negative trend).

There was also a significant increase in thyroid C-cell adenomas in the female rats (p_{Trend}=0.049) and a marginal increase[11] in adenomas and carcinomas combined (p_{Trend}=0.052) (Table 3). In males, the trend for adenomas was p_{Trend}=0.089 and for adenomas and carcinomas was p_{Trend} =0.097. Adenomas were seen in male rats at the interim sacrifice demonstrating that male rats at the interim sacrifice were at risk for this tumor. If these animals are added back into the analysis, the trend test in males has p_{Trend}=0.063 for adenomas and p_{Trend}=0.068 for adenomas and carcinomas combined.

There was a statistically significant increase in hepatocellular adenomas (p_{Trend}=0.015) and adenomas and carcinomas combined (p_{Trend}=0.050) but no increase in carcinomas alone (p_{Trend}=0.637) in male rats. There were no other histological findings in the liver.

Males also showed a statistically significant (p_{Trend}=0.042) increase in skin kertoacanthomas. There was also an increase in skin cysts with epidermal inclusion (p_{Trend}=0.043).

Several other tumors demonstrating significant findings in other studies of Sprague-

[11] In statistics, it is common to refer to p-values in the range of 0.10>p-value>0.05 as marginal when the target p-value is ≤0.05; this is done to avoid missing trends in data reflected by almost significant findings

Dawley rats are included in Table 3 and do not show significant effects.

In conclusion, the finding of an increased incidence of pancreatic islet-cell tumors in this study cannot easily be ruled out as a chance finding. Findings of significant increases in liver tumors in male rats with no increases in carcinomas needs to be compared with other studies. The findings of significant increases in thyroid c-cell tumors in males and females should be compared with other studies. The findings of skin keratoacanthomas in males and adrenal cortical carcinomas in females are fairly strong. This study will be included in the overall evaluation of causation.

Table 3: Tumors of interest in male and female Sprague-Dawley rats from the 24-month feeding study of Stout and Ruecker (1990)

Tumor	Doses (mg/kg/day) or Tumor Incidence[1]				Trend Test p-value
Males	0	89	362	940	
Testicular Interstitial Cell Tumors	2/50	0/50	3/50	2/50	0.296
Pancreas Islet Cell Adenomas	1/48	8/47*	5/50	7/49*	0.147
Pancreas Islet Cell Carcinomas	1/48	0/47	0/50	0/49	1.000
Pancreas Islet Cell Adenomas or Carcinomas	2/48	8/47*	5/50	7/49*	0.206 (<0.001)[2]
Thyroid C-cell Adenomas	0/50	4/48	8/48**	5/50*	0.089
Thyroid C-cell Carcinomas	0/50	2/48	0/48	1/50	0.442
Thyroid C-cell Adenomas and Carcinomas	0/50	6/50*	8/50**	6/50*	0.097
Thyroid Follicular-cell Adenomas	2/50	1/48	3/48	2/50	0.408
Thyroid Follicular-cell Carcinomas	0/50	0/48	0/48	1/50	0.255
Thyroid Follicular-cell Adenoma and Carcinoma	2/50	1/48	3/48	3/50	0.232
Hepatocellular Adenomas	3/50	2/50	3/50	8/50	0.015
Hepatocellular Carcinomas	3/50	2/50	1/50	2/50	0.637
Hepatocellular Adenomas and Carcinomas	6/50	4/50	4/50	10/50	0.050
Kidney Adenomas	0/50	2/50	0/50	0/50	0.813
Skin Keratoacanthomas	0/49	3/46	4/50	5/48	0.042
Skin Basal Cell Tumors	0/49	0/46	0/50	1/48	0.249
Females	0	113	457	1183	
Thyroid C-cell Adenomas	2/50	2/50	6/50	6/50	0.049
Thyroid C-cell Carcinomas	0/50	0/50	1/50	0/50	0.500
Thyroid C-cell Adenomas and Carcinomas	2/50	2/50	7/50	6/50	0.052
Adrenal Cortical Adenoma	1/50	2/50	2/50	1/50	0.603
Adrenal Cortical Carcinoma	0/50	0/50	0/50	3/50	0.015
Adrenal Cortical Adenoma and Carcinoma	1/50	2/50	2/50	4/50	0.090

This is publication [40]. 1 – Doses are given in the rows marked "Males" and "Females", tumor counts appear on the rows with the individual tumors; 2 – p_{hist} from Tarone's test; * $0.01<p\leq0.05$ for Fisher's Exact Test; ** $p\leq0.01$ for Fisher's Exact Test

Atkinson et al. (1993)[30] conducted a combined chronic toxicity/carcinogenicity study of glyphosate (98.9% pure). They used 50 Sprague-Dawley rats in each group for both sexes with dietary exposures given in Table 4. An additional 35 rats/sex/dose were included for interim sacrifices.

There were no survival differences in this study and there was no indication that the highest dose used exceeded the maximum-tolerated dose.

The authors reported no significant effects, as do **EPA**[54] and **EFSA**[55]. The study did not do detailed histopathological examination on all animals in all groups for every tumor type, but did examine all control and high dose animals, all animals that died before study termination and animals showing macroscopic tumors at study termination; liver, kidney and lungs were examined for all animals. This severely weakens the study for addressing dose-response trends. However, in reviewing the pathology tables, thyroid follicular adenomas and carcinomas were found to be marginally significant (p_{Trend}=0.099) by the trend test. If the three middle exposure groups had seen no other tumors and the denominators were the entire 50 animals on study, the trend analysis becomes significant (p_{Trend}=0.034). The study also had an increase in skin epitheliomas (p_{Trend}=0.047); these are the same as skin keratoacanthomas.

This study showed a marginal increase in thyroid follicular cell tumors and an increase in skin keratoacanthomas. Without examination of the animals free of gross tumors at terminal sacrifice, the findings from this study will be given less weight in the overall evaluation of causation.

Table 4: Tumors of interest in male and female Sprague-Dawley rats from the 24-month feeding study of Atkinson et al. (1993)

Tumor	Doses (mg/kg/day) or Tumor Incidence[1]					Trend Test p-value
Males	0	11	112	320	1147	
Testicular Interstitial Cell Tumors	3/50	1/25	0/19	0/21	2/50	0.580
Pancreas Islet Cell Adenomas	7/50	1/24	2/17	2/21	1/49	0.974
Pancreas Islet Cell Carcinomas	0/50	0/24	0/17	0/21	0/49	---
Pancreas Islet Cell Adenomas or Carcinomas	7/50	1/24	2/17	2/21	1/49	0.974
Thyroid C-cell Adenomas	9/50	1/21	1/17	2/21	8/49	0.278
Thyroid C-cell Carcinomas	0/50	0/21	0/17	1/21	1/49	0.178
Thyroid C-cell Adenomas and Carcinomas	9/50	1/21	1/17	2/21	9/49	0.197
Thyroid Follicular-cell Adenomas	0/50	0/21	0/17	1/21	2/49	0.067
Thyroid Follicular-cell Carcinomas	0/50	0/21	0/17	1/21	0/49	0.443
Thyroid Follicular-cell Adenoma and Carcinoma	0/50	0/21	0/17	2/21	2/49	0.099
Hepatocellular Adenomas	2/50	1/50	1/49	2/50	2/50	0.325
Hepatocellular Carcinomas	0/50	1/50	1/49	0/50	0/50	0.760
Hepatocellular Adenomas and Carcinomas	2/50	2/50	2/49	2/50	2/50	0.480
Kidney Adenomas	1/50	0/50	0/50	0/50	0/50	1.000
Skin Epithelioma (Keratoacanthomas)	1/50	2/25	0/19	0/21	5/50	0.047
Skin Basal Cell Tumors	1/50	0/25	0/19	0/21	0/50	1.000
Females	0	12	109	347	1134	
Thyroid C-cell Adenomas	8/50	1/27	1/29	2/29	7/49	0.207
Thyroid C-cell Carcinomas	0/50	0/27	0/29	0/29	0/49	---
Thyroid C-cell Adenomas and Carcinomas	8/50	1/27	1/29	2/29	7/49	0.207
Adrenal Cortical Adenoma	0/48	0/26	0/29	0/30	0/49	---
Adrenal Cortical Carcinoma	0/48	0/26	0/29	2/30	0/49	0.493
Adrenal Cortical Adenoma and Carcinoma	0/48	0/26	0/29	2/30	0/49	0.493

This is publication [30]. 1 – Doses are given in the rows marked "Males" and "Females", tumor counts appear on the rows with the individual tumors; * $0.01<p\leq0.05$ for Fisher's Exact Test; ** $p\leq0.01$ for Fisher's Exact Test

Brammer (2001)[31] conducted a two-year carcinogenicity study in Wistar rats in which groups of 52 animals were exposed to glyphosate (97.6% pure) at doses provided in Table 5 . An additional 12 animals were sacrificed at one-year.

A significant positive trend in survival was noted by the EPA (p=0.03), however this trend was not accomplished using a Kaplan-Meir test[142] (the appropriate test), but simply a test relating to the percent surviving to terminal sacrifice. There was no indication that the highest dose used exceeded the maximum-tolerated dose.

EPA[54], but not **EFSA**[55], noted there was a statistically significant trend of hepatocellular adenomas in male rats with the highest dose also being statistically significant from the control. Trend analysis gives p_{Trend}=0.008 and the Fisher's exact test comparison of high dose to control is p_{Fisher}=0.027. EPA dismissed this finding as potentially due to a slight difference in the number of animals at the terminal sacrifice in this study versus controls. However, no formal statistical evaluation of survival is provided and it cannot be assumed from these numbers that survival was significantly impacted in these animals. **Greim et al. (2015)**[58] used slightly different numbers for this tumor because three animals (one in the control group, one in the low-dose group and one in the mid-dose group) in the interim sacrifice group died before their sacrifice time and, from the pathology tables provided in their paper, these could not be separated from others. **Greim et al. (2015)**[58] dismissed these findings, partly because of the same survival argument used by the EPA and partly because they had a historical control dataset where the range of historical response was from 0-11.5%; they did not provide the mean response or the individual tumor responses for these historical controls. As mentioned earlier, dismissing results because they are in the range of the historical controls is an unacceptable method for using historical controls to evaluate a study, and in this case, there is no reason to question the concurrent controls.

Historical control data was obtained from 16 control groups in Wistar rats from Charles River Laboratories for the years 2003 to 2011 [143]. Although these are outside of the optimal time range for the animals used in the **Brammer (2001)** study, they can serve as an illustration of why using a range can be misleading. There were 52 liver adenomas seen in 1217 control animals for a mean response of 4.27% with a range of 0% to 17.5% (individual study findings of 6/100, 0/60, 1/60,1/50,1/80, 14/112, 1/65, 0/60, 21/120, 0/50, 1/50, 2/60, 0/50, 1/100, 1/150, 2/50; 13 studies with ≤2% response). Using these historical controls, Taron's test yields p_{Hist}=0.005 (Table 5). Thus, even though the responses seen in **Brammer (2001)** are in the range of the historical controls, the trend is highly significant when historical controls are used appropriately. **Greim et al. (2015)** also mentioned findings of increased toxicity at the high dose for which they provided numbers for only hepatocyte fat vacuolation and hepatitis; none of these findings were statistically significant by any test.

In conclusion, this study shows a positive result for hepatocellular adenomas in male Wistar rats and will be included in the overall evaluation of causation.

Table 5: Tumors of interest in male and female Wistar rats from the 24-month feeding study of Brammer (2001)

Tumor	Doses (mg/kg/day) or Tumor Incidence[1]				Trend Test p-value
Males	0	121	361	1214	
Hepatocellular Adenomas	0/53	2/51	0/53	5/52*	0.008
Hepatocellular Carcinomas	0/53	0/51	0/53	0/52	---
Hepatocellular Adenomas and Carcinomas	0/53	2/51	0/53	5/52*	0.008 (0.005)[2]
Pituitary Adenomas	16/53	15/52	18/53	18/52	0.277
Pituitary Carcinomas	0/53	0/52	0/53	0/52	---
Pituitary Adenomas and Carcinomas	16/53	15/52	18/53	18/52	0.277
Skin Keratoacanthomas	1/53	0/53	1/53	1/52	0.387
Adrenal Pheochromocytomas	8/53	5/53	7/52	5/52	0.721
Females	0	145	437	1498	
Mammary Gland Adenomas	1/51	2/51	0/52	0/53	0.941
Mammary Gland Adenocarcinomas	2/51	0/51	0/52	2/53	0.271
Mammary Gland Adenomas and Adenocarcinomas	3/51	2/51	0/52	2/53	0.590
Pituitary Adenomas	42/51	40/51	42/52	45/53	0.261
Pituitary Carcinomas	0/51	0/51	0/52	0/53	---
Pituitary Adenomas and Carcinomas	42/51	40/51	42/52	45/53	0.261

This is publication [31]. 1 – Doses are given in the rows marked "Males" and "Females", tumor counts appear on the rows with the individual tumors; 2 – p_{Hist} from Tarone's test; * $0.01<p\leq0.05$ for Fisher's Exact Test; ** $p\leq0.01$ for Fisher's Exact Test

Pavkov and Wyand (1987)[37] exposed Sprague-Dawley rats to glyphosate trimesium salt (sulfosate, 56.2% pure) in feed for two years. Eighty animals/sex were tested in the control, low-dose and mid-dose groups, and 90/sex were tested in the high dose group. Doses of 0, 4.2, 21.2 and 41.8 mg/kg/day were used in males and 0, 5.4, 27, and 55.7 mg/kg/day in females. This study showed no significant findings according to EPA[54]. No details were given beyond that simple statement and no others reported on this study. The doses in this study are far below the MTD so this study would have reduced sensitivity to detect an effect if one existed. This study also used a different chemical than the other Sprague-Dawley rat studies and is not comparable on that basis.

This study is not acceptable for use in the evaluation of causality due to the lack of details about the study.

Suresh, (1996)[41] exposed Wistar rats to glyphosate (96.8% pure) in feed for two years. Fifty animals/sex were tested in four exposure groups shown in Table 6.

There were no survival differences in this study and there was no indication that the highest dose used exceeded the maximum-tolerated dose.

EPA[54] concluded there were no tumors increased due to glyphosate exposure in this study and **EFSA**[55] concluded that, "[n]one of the significant microscopic changes, increased and decreased incidences (in liver, spleen, lymph nodes, adrenals, thymus, gonads, uterus, mammary gland) observed have shown dose relationship, hence appeared to be incidental and not related to the treatment with the test compound." (page 491). **Greim et al. (2015)**[58] provided data on hepatocellular adenomas and carcinomas in both sexes but none of these showed significant trends or pairwise tests (Table 6). However, there was another study with a strong significant trend in hepatocellular adenomas in Wistar rats[31] so these are also included in Table 6 for comparison. No other tumors were mentioned by any other group and an examination of the grouped pathology tables show an increase in mammary gland adenomas at the mid-dose (p_{Fisher}=0.017) but no significant trend (Table 6). However, there was another study with a strong significant trend in mammary gland adenomas and adenocarcinomas combined in Wistar rats [42] so these are also included in Table 6 for comparison. Also, adrenal pheochromocytomas showed a significant increase (p_{Trend}=0.048) in males. Like the **Atkinson et al. (1993)**[30] study, **Suresh (1996)** did not do full pathology on all of the animals in the interim exposure groups making interpretation of this study problematic.

This study showed an increase in adrenal pheochromocytomas in males and will be included in the overall evaluation of causation.

Table 6: Tumors of interest in male and female Wistar rats from the 24-month feeding study of Suresh (1996)

Tumor	Doses (mg/kg/day) or Tumor Incidence[1]				Trend Test p-value
Males	0	6.3	59.4	595.2	
Hepatocellular Adenomas	24/50	22/50	10/48	21/50	0.391
Hepatocellular Carcinomas	21/50	28/50	18/48	24/50	0.418
Hepatocellular Adenomas and Carcinomas	45/50	50/50	28/48	45/50	0.286
Pituitary Adenomas	3/49	4/30	3/31	5/49	0.376
Pituitary Carcinomas	0/49	1/30	0/31	0/49	0.692
Pituitary Adenomas and Carcinomas	3/49	5/30	3/31	5/49	0.454
Skin Keratoacanthomas	0/50	0/30	0/32	0/50	---
Adrenal Pheochromocytomas	13/50	5/33	7/36	17/50	0.048
Females	0	8.6	88.5	886	
Mammary Gland Adenomas	2/40	3/30	8/33*	5/48	0.539
Mammary Gland Adenocarcinomas	3/40	0/30	0/33	0/48	1.000
Mammary Gland Adenomas and Adenocarcinomas	5/40	3/30	8/33	5/48	0.729
Pituitary Adenomas	7/49	13/33	7/23	6/50	0.967
Pituitary Carcinomas	1/49	0/33	0/23	0/50	1.000
Pituitary Adenomas and Carcinomas	8/49	13/33	7/23	6/50	0.976

This is publication [41]. 1 – Doses are given in the rows marked "Males" and "Females", tumor counts appear on the rows with the individual tumors; * $0.01<p\leq0.05$ for Fisher's Exact Test; ** $p\leq0.01$ for Fisher's Exact Test

Enemoto (1997)[34] exposed Sprague-Dawley rats to glyphosate (95.7% pure) in feed for two years. Fifty animals/sex were tested in four exposure groups (see Table 7). In addition, 10 animals per exposure group were exposed for 1 year and another 10 for 18 months at which point they were sacrificed and examined. These interim sacrifice animals (1 year and 18 months) are excluded from the analysis.

There were no survival differences in this study and there was no indication that the highest dose exceeded the maximum-tolerated dose.

EPA and EFSA both found no significant changes in tumors in any group. **Greim et al. (2015)** again provide tables for a number of tumors, none of which show significant effects except for the incidence of kidney adenomas in male rats (p_{Trend}=0.004, Table 7). Examining the pathology tables revealed two additional tumors showing an increase in tumor incidence with dose in males; skin keratoacanthomas (p_{Trend}=0.029) and skin basal cell tumors (p_{Trend}=0.004). Others tumors in Table 7 are included for comparison

across other studies in Sprague-Dawley rats.

This study showed a significant increase in kidney adenomas and skin tumors in males and will be included in the overall evaluation of causation.

Table 7: Tumors of interest in male and female Sprague-Dawley rats from the 24-month feeding study of Enemoto (1997)

Tumor	Doses (mg/kg/day) or Tumor Incidence[1]				Trend Test p-value
Males	0	104	354	1127	
Testicular Interstitial Cell Tumors	3/49	2/50	0/50	2/50	0.594
Pancreas Islet Cell Adenomas	4/50	1/50	1/50	1/50	0.859
Pancreas Islet Cell Carcinomas	0/50	0/50	1/50	0/50	0.500
Pancreas Islet Cell Adenomas or Carcinomas	4/50	1/50	2/50	1/50	0.844
Thyroid C-cell Adenomas	4/50	9/49	2/49	5/50	0.631
Thyroid C-cell Carcinomas	2/50	0/49	1/49	1/50	0.565
Thyroid C-cell Adenomas and Carcinomas	6/50	9/49	3/49	6/50	0.642
Thyroid Follicular-cell Adenomas	3/50	1/49	1/49	0/50	0.966
Thyroid Follicular-cell Carcinomas	1/50	0/49	0/49	0/50	1.000
Thyroid Follicular-cell Adenoma and Carcinoma	4/50	1/49	1/49	0/50	0.986
Hepatocellular Adenomas	0/50	0/50	1/50	0/50	0.500
Hepatocellular Carcinomas	0/50	1/50	2/50	0/50	0.642
Hepatocellular Adenomas and Carcinomas	0/50	1/50	3/50	0/50	0.690
Kidney Adenomas	0/50	0/50	0/50	4/50	0.004
Skin Keratoacanthomas	3/50	3/50	0/50	7/50	0.029
Skin Basal Cell Tumors	0/50	0/50	0/50	4/50	0.004
Females	0	115	393	1247	
Thyroid C-cell Adenomas	4/50	7/49	7/49	2/50	0.912
Thyroid C-cell Carcinomas	0/50	0/49	0/49	0/50	---
Thyroid C-cell Adenomas and Carcinomas	4/50	7/49	7/49	2/50	0.912
Adrenal Cortical Adenoma	0/50	1/50	1/50	0/50	0.626
Adrenal Cortical Carcinoma	0/50	0/50	0/50	0/50	---
Adrenal Cortical Adenoma and Carcinoma	0/50	1/50	1/50	0/50	0.626

This is publication [34]. 1 – Doses are given in the rows marked "Males" and "Females", tumor counts appear on the rows with the individual tumors; * $0.01<p\leq0.05$ for Fisher's Exact Test; ** $p\leq0.01$ for Fisher's Exact Test

Takahashi (1999) [43] conducted a 24-month carcinogenicity study of glyphosate (purity not given) in groups of 50 male and female Fischer F-344 rats with dietary exposures of 0, 25, 201, and 1750 mg/kg/d (males) and 0, 29.7, 239, and 2000 mg/kg/d (females). The data for this study are not available. This study was only mentioned by **JMPR** [57] and showed body weight changes at the highest exposure which probably exceeded the MTD. No tumor data were provided although JMPR concluded there was no increased carcinogenicity.

Due to the lack of tumor data, this study will not be included in the overall evaluation of causality.

Wood et al. (2009)[42] exposed Wistar rats to glyphosate (94.7% to 97.6% pure) in feed for two years. Fifty-one animals/sex were tested in four exposure groups at doses shown in Table 8.

No survival differences were seen in this study.

EFSA[55] found no dose-related tumor increases while **EPA**[54] noted an increase in mammary gland adenomas and adenocarcinomas combined with p_{Trend}=0.062 for adenomas, p_{Trend}=0.042 for adenocarcinomas and p_{Trend}=0.007 for the combined tumors (Table 8). EPA concluded there was no progression from adenoma to adenocarcinoma and argued the increase was not glyphosate related. This conclusion is contradicted by the fact that 6 animals in control and the lower dose groups got carcinomas with no adenomas in any of the animals in these groups suggesting progression was either not noted by the pathologist or the adenomas in the low-dose animals were subsumed by the carcinomas as they grew. It seems likely that, in this case, mammary gland adenocarcinomas can arise without the presence of any adenomas.

Greim et al (2015)[58] also noted an increase in skin keratoacanthoma in males (p_{Trend}=0.030). Review of the pathology tables also identified a significant increase in pituitary tumors in both males and females in this study. In males, pituitary adenomas (p_{Trend}=0.045) were significantly increased and adenomas and carcinomas (p_{Trend}=0.059) were marginally increased with no increase in carcinomas. Similarly, in females, pituitary adenomas (p_{Trend}=0.014) were significantly increased and adenomas and carcinomas (p_{Trend}=0.017) were marginally increased with no increase in carcinomas. Other tumors are included in Table 8 for comparison.

This study showed an increase in mammary tumors in females, skin keratoacanthomas in males and pituitary tumors in both males and females; this study will be used in the evaluation of causality.

Table 8: Tumors of interest in male and female Wistar rats from the 24-month feeding study of Wood et al. (2009)

Tumor	Doses (mg/kg/day) or Tumor Incidence[1]				Trend Test p-value
Males	0	85.5	285.2	1077.4	
Hepatocellular Adenomas	0/51	2/51	1/51	1/51	0.418
Hepatocellular Carcinomas	1/51	0/51	0/51	0/51	1.000
Hepatocellular Adenomas and Carcinomas	1/50	2/51	1/51	1/51	0.610
Pituitary Adenomas	16/51	11/51	10/51	20/51	0.045
Pituitary Carcinomas	1/50	0/51	0/51	0/51	1.000
Pituitary Adenomas and Carcinomas	17/51	11/51	10/51	20/51	0.059
Skin Keratoacanthomas	2/51	3/51	0/51	6/51	0.030
Adrenal Pheochromocytomas	2/51	0/51	3/51	2/51	0.306
Females	0	104.5	348.6	1381.9	
Mammary Gland Adenomas	0/51	0/51	0/51	2/51	0.062
Mammary Gland Adenocarcinomas	2/51	3/51	1/51	6/51	0.042
Mammary Gland Adenomas and Adenocarcinomas	2/51	3/51	1/51	8/51*	0.007
Pituitary Adenomas	24/51	23/51	16/51	32/51	0.014
Pituitary Carcinomas	0/51	1/51	0/51	0/51	0.750
Pituitary Adenomas and Carcinomas	24/51	24/51	16/51	32/51	0.017

This is publication [42]. 1 – Doses are given in the rows marked "Males" and "Females", tumor counts appear on the rows with the individual tumors; * $0.01<p\leq0.05$ for Fisher's Exact Test; ** $p\leq0.01$ for Fisher's Exact Test

Excel (1997)[35] exposed Sprague-Dawley rats to glyphosate (purity not given) in feed for two years. Fifty-one animals/sex were tested in four exposure groups at doses of 0, 150, 780 and 1290 mg/kg/day in males and 0, 210, 1060 and 1740 mg/kg/day in females. **EPA**[54], **EFSA**[55] and **Greim et al. (2015)**[58] had concerns with the quality of this study, the characterization of the chemical being used and with tumor rates in this strain of animals being too low. The Supplemental Material from **Greim et al. (2015)** on this study shows no significant increase in any tumor and virtually all animals having no tumors in controls and treated animals.

This study is inadequate for use in deciding on causality for the same reasons given by the **EPA**, **EFSA** and **Greim et al. (2015)**.

Chruscielska, K. (2000)[33] exposed Wistar rats to glyphosate as a 13.8% solution (purity not given) in drinking water for two years. According to **Greim et al. (2015)**[58],

this appears to be the glyphosate formulation Perzocyd. Eighty-five animals/sex were tested in four exposure groups. The authors listed the doses as control, 300 mg/L, 900 mg/L and 2700 mg/L in drinking water. **Greim et al. (2015)**[58] estimated the intake of glyphosate to be 0, 1.9, 5.7 and 17 mg/kg/day for females and 0, 2.2, 6.5, and 19 mg/kg/day in males. There was a slight increase in malignant adenomas of the pituitary gland and an opposite decrease in pituitary adenomas suggesting no effect or potentially a promotional effect in which adenomas are promoted to carcinomas by glyphosate. No other increased tumor responses were reported in the manuscript. Because of the low exposures, this study is an inadequate challenge to the animals (the highest dose is far below the MTD). The reporting of this study is very limited and it the overall quality of the work cannot be evaluated.

This study is inadequate for use in deciding on causality.

Seralini, G. E., et al. (2014)[39] exposed Sprague-Dawley rats to the glyphosate formulation Roundup in drinking water for two years as part of a broader experiment on Roundup-Ready Corn. Ten animals/sex were tested in four exposure groups at doses of 0, 0.00005, 400 and 22500 mg/L in females. The authors reported an increase in the incidence of mammary gland tumors (mainly fibroadenomas and adenocarcinomas) in female rats with incidences of 5/10 for control and 9/10, 10/10, 9/10 (p_{Fisher}=0.016) in the low-, mid- and high-doses groups respectively. It is difficult to assess the quality of this study due to limited reporting on the histopathological descriptions of the tumors and the very small sample size.

This study will not be used in the evaluation of causality.

7.1.3 Joint Analysis of Rat Carcinogenicity Studies

Table 9 summarizes the significance for all tumors of interest in Sprague-Dawley rats. For ease of discussion, we will refer to **Lankas (1981)** as study A, **Stout and Ruecker (1990)** as study B, **Atkinson et al. (1993)** as study C and **Enemoto (1997)** as study D.

Testes interstitial cell tumors were significantly increased in study A; the other three studies were negative for this tumor. On the basis of statistical significance, these studies are inconsistent. To reject these findings based upon only 1/4 being positive is the same as rejecting a coin as being fair if, in four flips of the coin, the result is one head and three tails; it simply is not possible and there is a better way to address these findings. Given different doses and different sample sizes, we need to formally test for consistency in these studies by pooling the data using logistic regression as discussed above. Pooling testes interstitial tumors results for the four studies yields a p-value for trend that is clearly non-significant (p_{TrendA}=0.461). However, as noted above, study A was for 26 months and the other two were for 24 months; the tumors could be a result of the longer exposure period even though the dose is substantially lower in this study compared the remaining three studies in Sprague-Dawley rats. There are no non-neoplastic lesions seen in the testis in studies A, B and D. Study C saw a marginal increase (p=0.092) in interstitial cell hyperplasia of the testis.

Pancreas islet-cell tumors showed a statistically significant increase against historical

controls in study B but no trend in the remaining studies. The pooled analysis was not significant for adenomas (p_{TrendA}=0.849), carcinomas (p_{TrendA}=0.731) or their combination (p_{TrendA}=0.875). There are no dose-related increases in islet cell non-neoplastic findings in any of the four studies in male Sprague-Dawley rats.

Thyroid C-cell adenomas and carcinomas combined, in males, show marginally significant dose-response trends in study B but not in the remaining three studies. Pooling all four studies yields a non-significant trend of p_{TrendA}=0.175. Individually, thyroid C-cell adenomas and c-cell carcinomas were not significant in any study and neither were their pooled analyses. Reanalyzing data on non-neoplastic toxicity, Study C has a significant increase in focal C-cell hyperplasia (p=0.048) and no other studies have significant increases in C-cell hyperplasia.

Thyroid follicular-cell adenomas and carcinomas combined, in males, show a marginally significant dose-response trend in study C but not in the remaining three studies. Pooling all four studies yields no significant trend with p_{TrendA}=0.446. Individually, thyroid follicular-cell adenomas and follicular-cell carcinomas were not significant in any study and neither were their pooled analyses. No non-neoplastic endpoints show dose-related changes for thyroid follicular cells in any study.

Hepatocellular adenomas and hepatocellular carcinomas, in males, show a significant dose-response trend in study B but not in the remaining three studies. Pooling all four studies yields a significant trend for adenomas with p_{TrendA}=0.029 and a positive non-significant trend for adenomas and carcinomas combined (p_{TrendA}=0.144). Hepatocellular carcinomas, while marginally significant in study A were negative in the other studies and clearly negative when combined. After reanalysis of these studies for non-neoplastic toxicity, study A shows a significant increase in basophilic foci (p=0.029), study B did not report on these and studies C and D show non-significant trends with the pooled analysis for a common trend not significant (p=0.358). Study A has an increase in clear-cell foci (p=0.033), study C has a marginal increase in clear-cell foci (p=0.057) and study D is non-significant with the pooled analysis showing a marginally significant trend (p=0.073).

Kidney adenomas in males showed a significant trend in study D and no trend in the remaining three studies in Sprague-Dawley rats. Pooling the four studies p_{TrendA}=0.039, but with significant heterogeneity in the slopes between the studies (p=0.002). Removing the 26-month study (study A) yields a p-value for the three combined 24-month studies of p_{TrendA}=0.038 and no heterogeneity in slopes. The only non-neoplastic pathology in the kidney is an increase in lymphocytic infiltration (p=0.037) in study A.

Skin keratoacanthomas were statistically significantly increased in studies B, C and D and none of these tumors were reported in study A. It is not clear from the presentation of the results in study A if the researchers were looking for these tumors. The pooled analysis over all four studies is significant (p_{TrendA}<0.001) and for just the pooled 24-month studies (p_{TrendA}<0.001) with no indication of heterogeneity in either analysis. Focal hyperkeratosis is increased in both sexes (p≤0.001 – M; p=0.015 – F) in study D and shows a significant decrease in study C in males (p=0.004).

Skin basal-cell tumors were statistically significantly increased in study D, studies A and B saw only one animal with this tumor in the high dose and study C saw only one animal with this tumor in the control group. The pooled analysis over all four studies is significant ($p_{TrendA}<0.001$) but with significant heterogeneity in slopes because of the marginal negative trend in study C. Pooling just the 24-month studies yielded $p_{TrendA}=0.009$ but still with significant heterogeneity of the slopes.

Study A saw a significant increase in thyroid C-cell carcinomas in female rats exposed to glyphosate ($p_{Trend}=0.003$) and a marginal increase in C-cell adenomas and carcinomas combined ($p_{Trend}=0.072$, $p_{hist}=0.037$). Study B showed a significant increase in thyroid C-cell adenomas ($p_{Trend}=0.049$) and a marginal increase in adenomas and carcinomas combined ($p_{Trend}=0.052$). Study C showed a non-significant positive response and study D was negative. The pooled analysis using all four studies yields $p_{TrendA}=0.287$ for adenomas, $p_{TrendA}=0.385$ for carcinomas and $p_{TrendA}=0.275$ for the combined tumors. This pooled analysis does not support the results seen in Study A. However, study A was for 26 months and the other three were for 24 months; the C-cell carcinomas could be a result of the longer exposure period even though the dose is substantially lower in this study compared to the other two. There are no non-neoplastic changes in thyroid C-cells in females in these studies.

Study B showed a significant increase in adrenal cortical carcinomas in female rats ($p_{Trend}=0.015$) and a marginal increase ($p_{Trend}=0.090$) in the combined adenomas and carcinomas. Study C showed a very slight increase in cortical adenomas while study A had basically flat dose response and study D saw no tumors of this type. The pooled analysis for adrenal cortical carcinomas is significant ($p_{Trend}=0.031$) but there is no trend for the adenomas ($p_{Trend}=0.713$) or the combined adenomas and carcinomas ($p_{Trend}=0.195$). Study A had a much greater number of cortical adenomas in control and low-dose animals than the other studies, but the pooled analysis without this study did not measurably change the results. Focal cortical hypertrophy shows a dose-related significant increase in studies A (p=0.048) and C (p=0.027), study B did not report hypertrophy independent of hyperplasia (the combined counts showed no increased dose-response), and study D did not report hypertrophy. There are no other dose-related increases in injury to adrenal cortical tissue in any of the studies.

Table 9: P-values for the Cochran-Armitage trend test and pooled logistic regression analysis for tumors with at least one significant trend test or Fisher's exact test (p≤0.05) in male and female Sprague-Dawley rats

Tumor	Individual study p-values for trend[1]				Common Trend	Heterogeneity Test
Males	A	B	C	D		
Testicular Interstitial Cell Tumors	**0.009**	0.296	0.580	0.594	0.461	0.105
Pancreas Islet Cell Adenomas	0.512	0.147 (**0.007**)[3]	0.974	0.859	0.849	0.143
Pancreas Islet Cell Carcinomas	0.251	1.000	---[2]	0.500	0.731	0.166
Pancreas Islet Cell Adenomas or Carcinomas	0.316	0.206	0.974	0.844	0.875	0.185
Thyroid C-cell Adenomas	0.743	0.089	0.278	0.631	0.210	0.532
Thyroid C-cell Carcinomas	0.505	0.442	0.495	0.565	0.322	0.898
Thyroid C-cell Adenomas and Carcinomas	0.748	0.097	0.197	0.642	0.175	0.526
Thyroid Follicular-cell Adenomas	0.122	0.408	0.067	0.966	0.464	0.055
Thyroid Follicular-cell Carcinomas	---[2]	0.255	0.443	1.000	0.448	0.137
Thyroid Follicular-cell Adenoma and Carcinoma	0.122	0.232	0.099	0.986	0.446	0.031
Hepatocellular Adenomas	0.471	**0.015**	0.325	0.500	**0.029**	0.664
Hepatocellular Carcinomas	0.062	0.637	0.760	0.642	0.803	0.269
Hepatocellular Adenomas and Carcinomas	0.173	**0.050**	0.480	0.690	0.144	0.428
Kidney Adenomas	0.938	0.813	1.000	**0.004**	**0.039**	0.002
Skin Keratoacanthomas	---[2]	**0.042**	**0.047**	**0.029**	**<0.001**	0.998
Skin Basal Cell Tumors	0.251	0.249	1.000	**0.004**	**<0.001**	0.009
Females	A	B	C	D		
Thyroid C-cell Adenomas	0.679	**0.049**	0.207	0.912	0.287	0.150
Thyroid C-cell Carcinomas	**0.003** (**<0.001**)[3]	0.500	---[2]	---[2]	0.385	0.041
Thyroid C-cell Adenomas and Carcinomas	0.072 (**0.037**)[3]	0.052	0.207	0.912	0.275	0.071
Adrenal Cortical Adenoma	0.851	0.603	---[2]	0.626	0.713	0.750
Adrenal Cortical Carcinoma	0.386	**0.015**	0.493	---[2]	**0.031**	0.199
Adrenal Cortical Adenoma and Carcinoma	0.801	0.090	0.493	0.626	0.195	0.520

[1] – Study A is Lankas (1981) [Table 2], Study B is Stout and Ruecker (1990) [Table 3], Study C is Atkinson et al. (1993) [Table 4] and Study D is Enemoto (1997) [Table 7]; [2] – three dashes "---" indicates all tumor counts are zero; [3] – using historical control data (see text for details) and Tarone's test

Table 10 summarizes the significance for all tumors of interest in Wistar rats. For ease of discussion, we will refer to **Suresh (1996)** as study E, **Brammer (2001)** as study F, and **Wood (2009)** as study G.

Study F saw a significant increase in hepatocellular adenomas and adenomas and carcinomas combined in male Wistar rats with increasing dose (p_{Trend}=0.008, p_{Hist}=0.005). The other two acceptable studies in Wistar rats did not see significant increases in these tumors nor did any study see a significant increase in carcinomas alone. Study E saw 48% response for hepatocellular adenomas in controls whereas the other two studies saw no tumors in the control animals. Thus, although all three studies are in Wistar rats, Study E has a significantly different control response from the other two. Study E did not give a substrain for the Wistar rats used, but studies F and G used different substrains. All three studies used different diets and were conducted in different facilities. Thus, there is no obvious explanation for the dramatically different rates in Study E**.** It is known that the same strain of rats from different laboratories can have markedly different control tumor responses. Even with these differences in control response, the analysis of the pooled studies yields p_{TrendA}=0.048 for adenomas, p_{TrendA}=0.492 for carcinomas and p_{TrendA}=0.029 for adenomas and carcinomas combined supporting the conclusion that glyphosate causes hepatocellular adenomas in Wistar rats. There is a significant decrease in basophilic-cell foci in study E (p=0.023), no foci at all in study F and no trend in study G. Clear-cell foci are not impacted by glyphosate in male Wistar rats.

Pituitary adenomas were significantly increased in males (p_{Trend}=0.045) and females (p_{Trend}=0.014) in study G. Study G also saw a marginal increase in adenomas and carcinomas combined in males (p_{Trend}=0.059) and a significant increase for this combination in females (p_{Trend}=0.017) but saw no increase in carcinomas alone in either sex. Studies E and F saw non-significant increases in adenomas in males with study E again showing a markedly different control response. In all three studies, there were only 2 animals with carcinomas in males, one in the controls for study G and the low dose for study E. In females, study E showed no trend for pituitary adenomas, study F showed a non-significant increase with Study E having a much smaller control response (17%) than study F (82%) with study G being in between (47%). As for males, there were only 2 pituitary carcinomas in the three studies, one in the controls for study E and the low-dose for study G. The pooled analysis in males was marginally significant for both adenomas (p_{TrendA}=0.057) and adenomas and carcinomas combined (p_{TrendA}=0.073) while in females, the pooled analyses were not significant (p_{TrendA}=0.105 and 0.129 respectively). There are no dose-dependent increases in any non-neoplastic lesion in male or female Wistar rats in any of the three studies.

Skin keratoacanthomas were not mentioned in study E (it was assumed there were none), showed a non-significant slight trend in study F and were significant (p_{Trend}=0.030) in study G. Controls were not very different and the pooled analysis was

significant (p_{TrendA}=0.032). No non-neoplastic pathologies are significantly linked to dose in the skin.

Adrenal pheochromocytomas were significantly increased in study E (p_{Trend}=0.048) but demonstrated no trend in the other two studies. Controls in study E had a rate of 26% for this tumor, study F 15% and study G 4%. The pooled analysis was not significant. There are no significant trends in non-neoplastic findings in any of the three studies.

Study G saw a significant increase in mammary gland carcinomas (p_{Trend}=0.042) and adenomas and adenocarcinomas combined (p_{Trend}=0.007) and a marginally significant trend for adenomas (p_{Trend}=0.062) in females. The other two studies were non-significant for these tumors but similar control responses. The pooled analysis of all three studies yields p_{TrendA}=0.448 for adenomas, p_{TrendA}=0.071 for adenocarcinomas, and p_{TrendA}=0.113 for adenomas and adenocarcinomas combined. There was considerable heterogeneity in the slopes from these three studies. Hyperplasia in mammary tissue is examined in all three studies with no significant findings in any study.

Table 10: P-values for the Cochran-Armitage trend test and pooled logistic regression analysis for tumors with at least one significant trend test or Fisher's exact test (p≤0.05) in male and female Wistar rats

Tumor	Individual study p-values for trend[1]			Common Trend	Homogeneity Test
Males	E	F	G		
Hepatocellular Adenomas	0.391	**0.008**	0.418	**0.048**	0.156
Hepatocellular Carcinomas	0.418	---[2]	1.000	0.492	0.242
Hepatocellular Adenomas and Carcinomas	0.286	**0.008**	0.610	**0.029**	0.194
Pituitary Adenomas	0.376	0.277	**0.045**	0.057	0.664
Pituitary Carcinomas	0.692	---[2]	1.000	0.771	0.956
Pituitary Adenomas and Carcinomas	0.454	0.277	0.059	0.073	0.700
Skin Keratoacanthomas	---[2]	0.387	**0.030**	**0.032**	0.823
Adrenal Pheochromocytomas	**0.048**	0.721	0.306	0.273	0.210
Females	E	F	G		
Mammary Gland Adenomas	0.539	0.941	0.062	0.448	0.015
Mammary Gland Adenocarcinomas	1.000	0.271	**0.042**	0.071	0.008
Mammary Gland Adenomas and Adenocarcinomas	0.729	0.590	**0.007**	0.113	0.064
Pituitary Adenomas	0.967	0.261	**0.014**	0.105	0.023
Pituitary Carcinomas	1.000	---	0.750	0.748	0.491
Pituitary Adenomas and Carcinomas	0.976	0.261	**0.017**	0.129	0.019

[1] – Study E is Suresh (1996) [Table 6], Study F is Brammer (2001) [Table 5], and Study G is Wood et al. (2009) [Table 8]; [2] – three dashes "---" indicates all tumor counts are zero

7.1.4 Analysis of Individual Mouse Studies

Reyna and Gordon (1974)[49] exposed Swiss White mice to glyphosate (>97% purity) in feed for 16 months in males and 18 months in females. Fifty animals/group/sex were tested in three exposure groups; control, 17 mg/kg and 50 mg/kg. Only 10 animals per group were examined for histopathological changes.

There was no impact on survival of administration of glyphosate and no indication that the high dose exceeded the MTD.

No significant increases were seen in any tumor from this study. Because of inconsistencies in the data from this study, EPA concluded it was invalid [139] yet still used it as evidence of no carcinogenic effect of glyphosate [140]. Regardless, given the small sample size for histopathological evaluation and the low doses used for this study, this study is inadequate.

This study will not be used in the evaluation of causality.

Knezevich and Hogan, (1983)[46] exposed CD-1 mice to glyphosate (99.8% pure) in feed for two years. Fifty animals/group/sex were tested in four exposure groups (see Table 11).

There were no survival differences in this study and there was no indication that the highest dose used exceeded the MTD.

EPA[144] found a significant increase in kidney tubular cell adenomas in male mice based upon the original pathology done from the study (p_{Trend}=0.019). Kidney tubular cell adenomas are very rare tumors in CD-1 mice so it is important to compare these results with the historical controls. EPA was given historical controls for glyphosate that they used to evaluate these kidney adenomas [145]. Using these data results in p_{Hist}=0.002. In their recent review, **EPA** [140] used this same historical control database. EPA originally used a similar analysis and concluded the kidney tumors were an oncogenic response. However, in 1985, the registrant had a group of pathologists review the kidney slides. Using additional kidney sections from this study, the pathologists identified an additional adenoma in the control animals and changed the classification for three adenomas to carcinomas (Table 11). With these changes, the adenomas no longer have a significant trend (P_{Trend}=0.442, P_{Hist}=0.138) but carcinomas have a marginally significant trend against concurrent controls and a clearly significant trend using historical controls (p_{Trend}=0.063, $p_{Hist}\leq$0.001). These historical control rates may not apply to this analysis because the reevaluation of the kidney tumors considered additional sections and no information is available on how additional sections affect historical control rates in this strain of mice; differences have been seen in other settings[146]. The incidence of combined carcinomas and adenomas has the same marginal significance against the concurrent control and significance against the historical controls (p_{Trend}=0.065, p_{Hist}=0.008) .

The **EPA**[54] has produced many different arguments to dismiss the findings of renal tumors from this study. One argument is that the pathology working group requested

by the EPA in 1986 concluded these lesions were not glyphosate related because "*1) renal tubular cell tumors are spontaneous lesions for which there is a paucity of historical control data for this mouse stock; 2) there was no statistical significance in a pairwise comparison of treated groups with the concurrent controls and there was no evidence of a statistically significant linear trend; 3) multiple renal tumors were not found in any animal; and 4) compound-related nephrotoxic lesions, including pre-neoplastic changes, were not present in male mice in this study*." Reason number one no longer exists as EPA themselves provide a good historical control databases for CD-1 mice [140]. The second reason, while technically correct, is not supportable since the Agency's own guidelines for evaluating carcinogenicity studies state that *"Significance in either kind of test* [trend or pair-wise] *is sufficient to reject the hypothesis that chance accounts for the result."* The third reason is also weak since one would not expect (nor require) multiple tumors to appear when dealing with a rare tumor. For the fourth point, EPA provides data on the rate of bilateral chronic interstitial nephritis in the study which it considers to show no statistically significant results although the trend test is highly significant (p_{Trend}=0.006). EPA then states, without reference, that "*chronic interstitial nephritis is not considered to be a precursor lesion for tubular neoplasms*". No published research exists to either support or refute this statement. However, chronic interstitial nephritis is an inflammation of the interstitial tissue surrounding the glomeruli and tubules in the kidney. Inflammation is well known to play an important role in kidney cancer[147] and many other cancers so this argument also fails to support rejection of these findings.

This study also saw an increase (p_{Trend}=0.016) in composite lymphosarcomas in the spleen in females. Composite lymphosarcoma is an old designation for malignant lymphomas. BFR [148] provided numbers for malignant lymphomas for males and females in this study; using these numbers the resulting trend is marginal in females (p_{Trend}=0.070) and negative in males (p_{Trend}=0.754).

Other CD-1 mouse studies have seen increases in various tumors which are also given in Table 11 for comparison purposes..

In summary, this study shows a positive result for kidney tumors in male CD-1 mice and composite lymphosarcomas in the spleen in female mice and will be included in the overall evaluation of causation.

Table 11: Tumors of interest in male and female CD-1 mice from the 24-month feeding study of Knezevich and Hogan (1983)

Tumor	Doses (mg/kg/day) or Tumor Incidence[1]				Trend Test p-value
Males	0	157	814	4841	
Kidney Adenomas (original pathology)	0/49	0/49	1/50	3/50	0.019 (0.002)[4]
Kidney Adenomas[2]	1/49	0/49	0/50	1/50	0.442 (0.138)[4]
Kidney Carcinomas[2]	0/49	0/49	1/50	2/50	0.063 (0.001)[4]
Kidney Adenomas and Carcinomas[2]	1/49	0/49	1/50	3/50	0.065 (0.008)[4]
Malignant Lymphomas[3]	2/49	5/49	4/50	2/50	0.754
Hemangiosarcomas[3]	0/49	0/49	1/50	0/50	0.505
Alveolar-Bronchiolar Adenomas	5/48	9/50	9/50	9/50	0.294
Alveolar-Bronchiolar Carcinomas	4/48	3/50	2/50	1/50	0.918
Alveolar-Bronchiolar Adenomas and Carcinomas	9/48	12/50	11/50	10/50	0.576
Females	0	190	955	5874	
Hemangiomas	0/49	1/49	1/50	0/50	0.631
Harderian Gland Adenomas	2/45	0/48	1/49	0/44	0.877
Harderian Gland Carcinomas	0/45	0/48	0/49	0/44	---
Harderian Gland Adenomas and Carcinomas	2/45	0/48	1/49	0/44	0.877
Alveolar-Bronchiolar Adenomas	10/49	9/50	10/49	1/50	0.999
Alveolar-Bronchiolar Carcinomas	1/49	3/50	4/49	4/50	0.183
Alveolar-Bronchiolar Adenomas and Carcinomas	11/49	12/50	14/49	5/50	0.985
Spleen Composite Lymphosarcoma	1/50	1/48	1/49	5/49	0.016
Malignant Lymphomas	5/49	6/49	6/49	10/49	0.070

This is publication [46]. 1 – Doses are given in the rows marked "Males" and "Females", tumor counts appear on the rows with the individual tumors; 2 – tumor counts obtained from **EPA (2017)** [140]; 3 – tumor counts obtained from EFSA [148]; * $0.01<p\leq0.05$ for Fisher's Exact Test; ** $p\leq0.01$ for Fisher's Exact Test; 4 – pHist from Tarone's test

Atkinson, et al., (1993)[44] exposed CD-1 mice to glyphosate (>97% purity) in feed for two years. Fifty animals/group/sex were tested in four exposure groups (see Table 12).

There was no impact on survival of administration of glyphosate and no indication that the high dose exceeded the MTD.

Hemangiosarcomas were the only tumors showing a significant trend in this study (P_{Trend}=0.004). There is a marginal trend for malignant lymphomas (P_{Trend}=0.087).

The **EPA[54]** concluded the findings for hemangiosarcomas in this study were not treatment related based upon the tumors appearing only in the high dose group, a lack of statistical significance between the response in this group and control response and that these tumors are commonly observed in mice as both spontaneous and treatment related effects. There is no scientific support for excluding positive findings in the highest dose group, a view also held by the **SAP**[121]. As mentioned earlier, EPA's guidelines treat trend tests and Fisher's Exact test results as equal justification of a positive response, although in this case, the value of the comparison of the highest exposure group to controls, p_{Fisher}=0.059, is marginally significant. The argument regarding the frequency of this tumor in controls can be addressed directly by the evaluation against historical control rates. Using the historical control database in CD-1 mice from Charles River Laboratories [149], P_{Hist} <0.001 with a mean historical control incidence of hemangiosarcomas in controls from two-year cancer bioassays in CD-1 mice of 2.5%. The **SAP**[121] stated very clearly that the practice, being used by the EPA, of negating a positive finding because of historical control data was not acceptable[121] (page 63). The EPA Cancer Guidelines[3] state this very clearly "*…statistically significant increases in tumors should not be discounted simply because incidence rates in the treated groups are within the range of historical controls or because incidence rates in the concurrent controls are somewhat lower than average.*"

Female mice showed a significant increase in lung alveolar-bronchiolar adenomas and carcinomas combined (p=0.048) and non-significant increases in adenomas (p=0.0144) and carcinomas (p=0.110) individually.

Other CD-1 mouse studies have seen increases in various tumors which are also given in Table 12 for comparison purposes.

In summary, this study shows a positive result for hemangiosarcomas and a marginal result for malignant lymphomas in male CD-1 mice and will be included in the overall evaluation of causation.

Table 12: Tumors of interest in male and female CD-1 mice from the 24-month feeding study of Atkinson et al. (1993)

Tumor	Doses (mg/kg/day) or Tumor Incidence[1]				Trend Test p-value
Males	0	98	297	988	
Kidney Adenomas	1/50	1/50	0/50	0/50	0.938
Kidney Carcinomas	1/50	1/50	0/50	0/50	0.938
Kidney Adenomas and Carcinomas	2/50	2/50	0/50	0/50	0.981
Malignant Lymphomas[2]	4/50	2/50	1/50	6/50	0.087
Hemangiosarcomas[2]	0/50	0/50	0/50	4/50	0.004 (<0.001)[4]
Alveolar-Bronchiolar Adenomas	12/50	15/50	12/50	16/50	0.231
Alveolar-Bronchiolar Carcinomas	10/50	7/50	8/50	9/50	0.456
Alveolar-Bronchiolar Adenomas and Carcinomas	22/50	22/50	20/50	25/50	0.231
Females	0	102	298	1000	
Hemangiomas	0/50	0/50	0/50	0/50	---
Harderian Gland Adenomas[3]	0/50	0/50	0/50	0/50	---
Harderian Gland Carcinomas[3]	0/50	0/50	0/50	0/50	---
Harderian Gland Adenomas and Carcinomas[3]	0/50	0/50	0/50	0/50	---
Alveolar-Bronchiolar Adenomas	7/50	5/49	3/50	9/50	0.144
Alveolar-Bronchiolar Carcinomas	3/50	2/49	1/50	5/50	0.110
Alveolar-Bronchiolar Adenomas and Carcinomas	10/50	7/49	4/50	14/50	0.048
Malignant Lymphomas[2]	14/50	12/50	9/50	13/50	0.484

This is publication [138]. 1 – Doses are given in the rows marked "Males" and "Females", tumor counts appear on the rows with the individual tumors; 2 – tumor counts obtained from **EFSA** [148]; 3 – No tumors were listed for this tissue in females, but there were no animals listed as evaluated histopathologically in this tissue; * $0.01<p\leq0.05$ for Fisher's Exact Test; ** $p\leq0.01$ for Fisher's Exact Test; 4 – pHist from Tarone's test

Sugimoto (1997)[50] exposed CD-1 mice to glyphosate (94.61-95.67% pure) in feed for two years. Fifty animals/group/sex were tested in four exposure groups (see Table 13).

There were no effects of treatment on survival and no indication the highest dose had exceeded the MTD.

Kidney adenomas were mariginally increased ($p_{Trend}=0.062$) in male mice with two adenomas in the highest exposure group. In the historical control database for CD-1 mice developed by **Giknis and Clifford (2000)**, they saw 7 kidney adenomas in 5 of the

control groups terminated at 18 months (26 studies) or 24 months (20 studies) with the highest response being 4%. This means there are 3 studies with 1 tumor each and 2 studies with 2 tumors each. They did not give individual tumor counts, but using the worst scenario of putting all 7 studies in the 18—month control groups yields p_{Hist}=0.009, a strong positive finding.

Malignant lymphomas (p_{Trend}=0.016) and hemangiosarcomas (p_{Trend}=0.062) in male mice and hemangiomas (p_{Trend}=0.002) and Harderian gland adenomas (p_{Trend}=0.040) in female mice all showed increased tumor incidence with increasing dose. This study also had an increase in animals with any malignancy in males (p_{Trend}=0.001) but not in females (p_{Trend}=0.362). Note that no hemangiosarcomas were seen in the 26 control groups evaluated by **Giknis and Clifford (2000)** and yields p_{Hist}=0.005. The fact that this tumor was never seen in the historical controls should strongly support any positive finding as being significant.

EPA[54] only addressed the hemangiomas in the female mice and did not note any other significant effects. For the females, EPA argued that the high dose was approximately four times higher than the current recommended high dose from the **OECD guidelines**[150]. This study was correctly designed under the previous guidelines (the limit was <5% in feed) and there is no indication that this dose exceeded the MTD. The EPA also argued that when the p-value for Fisher's Exact test was adjusted for multiple comparisons, the new p-value for the high-dose group for hemangiomas was 0.055.

Other CD-1 mouse studies have seen increases in various tumors which are also given in Table 13 for comparison purposes..

In summary, this study shows a positive result for kidney adenomas, malignant lymphomas and hemangiosarcomas in male CD-1 mice, hemangiomas and Harderian gland adenomas in female CD-1 mice and an overall increase in malignancies as a function of exposure in male CD-1 mice. This study will be included in the overall evaluation of causation.

Table 13: Tumors of interest in male and female CD-1 mice from the 18-month feeding study of Sugimoto (1997)

Tumor	Doses (mg/kg/day) or Tumor Incidence[1]				Trend Test p-value
Males	0	165	838.1	4348	
Kidney Adenomas	0/50	0/50	0/50	2/50	0.062 (0.009)[3]
Kidney Carcinomas	0/50	0/50	0/50	0/50	---
Kidney Adenomas and Carcinomas	0/50	0/50	0/50	2/50	0.062
Malignant Lymphomas[2]	2/50	2/50	0/50	6/50	0.016
Hemangiosarcomas[2]	0/50	0/50	0/50	2/50	0.062 (0.005)[3]
Alveolar-Bronchiolar Adenomas	8/50	14/50	13/50	11/50	0.513
Alveolar-Bronchiolar Carcinomas	1/50	1/50	6/50	4/50	0.148
Alveolar-Bronchiolar Adenomas and Carcinomas	9/50	15/50	19/50*	15/50	0.294
Females	0	153.2	786.8	4116	
Hemangiomas	0/50	0/50	2/50	5/50*	0.002
Harderian Gland Adenomas	1/50	3/50	0/50	5/50	0.040
Harderian Gland Carcinomas	0/50	0/50	0/50	0/50	---
Harderian Gland Adenomas and Carcinomas	1/50	3/50	0/50	5/50	0.040
Alveolar-Bronchiolar Adenomas	8/50	5/50	12/50	5/50	0.800
Alveolar-Bronchiolar Carcinomas	1/50	2/50	3/50	1/50	0.623
Alveolar-Bronchiolar Adenomas and Carcinomas	9/50	7/50	15/50	6/50	0.842
Malignant Lymphomas[2]	6/50	4/50	8/50	7/50	0.294

This is publication [50]. 1 – Doses are given in the rows marked "Males" and "Females", tumor counts appear on the rows with the individual tumors; 2 – tumor counts obtained from **EFSA** [148]; * $0.01<p\leq0.05$ for Fisher's Exact Test; ** $p\leq0.01$ for Fisher's Exact Test; 3 – pHist from Tarone's test

Wood et al., (2009)[51] exposed CD-1 mice to glyphosate (95.7% pure) in feed for 80 weeks. Fifty-one animals/groups/sex were tested in four exposure groups (see Table 14).

There was no effect on survival and no information suggesting the study exceeded the MTD.

There was a monotonic increase in lung adenocarcinomas ($p_{Trend}=0.028$) in males and a

monotonic increase in malignant lymphomas (p_{Trend}=0.007) in males.

For lung adenocarcinomas, the **EPA** [140] again argued a lack of significance for pairwise comparisons (in violation of its guidelines) and that there was no evidence of progression from adenomas to carcinomas. Even though there was no increase in lung adenomas as a function of exposure, it is possible to have an increase in lung adenocarcinomas without an associated increase in adenomas[151].

For malignant lymphomas, **EPA** notes that there was a statistically significant response and that the high dose was significantly different from control (p_{Fisher}=0.028), but then uses an argument based upon the number of analyses done in this study to adjust the Fisher Exact test p-value to 0.082 (an adjustment for multiple comparisons is indeed warranted in evaluating the outcomes of these animal cancer studies, this will be addressed later in my report in the evaluation of all of the studies combined). The **EPA** [140] also used historical control data[152, 153] to exclude the malignant lymphomas and cite a mean response of 4.5% and a range of 1.5% to 21.7%. **Son and Gopinath (2004)**[153] saw 21 animals out of 1453 examined prior to 80 weeks with lung adenocarcinomas (1.4%). **Giknis and Clifford (2005)** [152] saw a mean rate of 4.5% with a range of 0% to 21.7% in 52 studies which included mostly 78 week controls (26 studies) and 104 week controls (26 studies). Including only studies of 80 weeks or less, the rate in **Giknis and Clifford (2005)** is 37/1372=2.7% with a range of 0% to 14%. Using this historical control database yields p_{Hist}=0.003, directly in contradiction to their contention that this database eliminates the significant finding. **Giknis and Clifford (2000)**[149] did a similar evaluation, using mostly the same data as their 2005 paper and saw an average tumor incidence before 80 weeks of 2.6% with a range of 0% to 14%. Based upon its flawed interpretation of the **Giknis and Clifford (2005)** historical controls, **EPA** argues that the incidence of concurrent controls in the study was low (it was 0%) and rejected the positive finding. In fact, of the 26 studies in the 18-month control groups evaluated by **Giknis and Clifford (2005),** eight (31%) had response of 0% and eight (31%) had only one tumor. The evaluation used by the EPA is incorrect. In addition, as noted earlier, the use of historical control data to negate a positive finding is not supported by **EPA's guidelines**[3, 121] or its **SAP**[121].

There was an increase in the number of animals with multiple malignant tumors (P_{Trend}=0.046)

Other CD-1 mouse studies have seen increases in various tumors which are also given in Table 14 for comparison purposes..

In summary, this study shows a positive result for malignant lymphomas and lung adenocarcinomas in male CD-1 mice and will be included in the overall evaluation of causation.

Table 14: Tumors of interest in male and female CD-1 mice from the 18-month feeding study of Wood et al. (2009)

Tumor	Doses (mg/kg/day) or Tumor Incidence[1]				Trend Test p-value
Males	0	71.4	234.2	810	
Kidney Adenomas	0/51	0/51	0/51	0/51	---
Kidney Carcinomas	0/51	0/51	0/51	0/51	---
Kidney Adenomas and Carcinomas	0/51	0/51	0/51	0/51	---
Malignant Lymphomas[2]	0/51	1/51	2/51	5/51*	0.007
Hemangiosarcomas[2]	0/51	0/51	0/51	0/51	---
Alveolar-Bronchiolar Adenomas	9/51	7/51	9/51	4/51	0.924
Alveolar-Bronchiolar Carcinomas	5/51	5/51	7/51	11/51	0.028
Alveolar-Bronchiolar Adenomas and Carcinomas	14/51	12/51	16/51	15/51	0.336
Females	0	97.9	299.5	1081.2	
Hemangiomas	0/51	2/51	0/51	1/51	0.438
Harderian Gland Adenomas	1/51	0/51	0/51	2/51	0.155
Harderian Gland Carcinomas	2/51	0/51	0/51	0/51	1.000
Harderian Gland Adenomas and Carcinomas	3/51	0/51	0/51	2/51	0.372
Alveolar-Bronchiolar Adenomas	2/51	4/51	2/51	2/51	0.656
Alveolar-Bronchiolar Carcinomas	5/51	2/51	2/51	3/51	0.601
Alveolar-Bronchiolar Adenomas and Carcinomas	7/51	6/51	4/51	5/51	0.688
Malignant Lymphomas[2]	11/51	8/51	10/51	11/51	0.353

This is publication [51]. 1 – Doses are given in the rows marked "Males" and "Females", tumor counts appear on the rows with the individual tumors; 2 – tumor counts obtained from **EFSA** [148]; * $0.01<p\leq0.05$ for Fisher's Exact Test; ** $p\leq0.01$ for Fisher's Exact Test

Takahashi (1999) [52] exposed groups of 50 male and female CD-1 mice to glyphosate (97.5% purity) for 18 months at doses shown in Table 15. The original report on this study was not available. The only data available came from **JMPR (2017)** [57] and they only reported on kidney tumors in males and malignant lymphomas in females.

JMPR (2017) did not specify that the study was OECD compliant, but the laboratory doing the study is OECD certified and the description of the study appears to follow OECD guidance. The highest exposure group showed signs of digestive toxicity (loose stools, anal prolapse) and showed significant difference in mortality post 26 weeks of exposure (test not specified) in males and females. There were also food consumption issues and decreased weight gain in the highest exposure group for both sexes suggesting this group exceeded a maximum tolerated dose.

Kidney adenomas (p_{Trend}=0.019) and kidney adenomas and carcinomas combined (p_{Trend}=0.005) but not carcinomas alone (p_{Trend}=0.250) were significantly increased in males in this study. Malignant lymphomas were significantly increased (p_{Trend}=0.050) in females. No other information on tumors from this study were available. Removing the highest dose because of potential exceedance of the MTD removes these significant findings.

According to **JMPR (2017)**, "These tumours were re-examined by the original study pathologist in 2012 because the Pesticide Expert Panel, Food Safety Commission of Japan requested more information on historical control data and association with the non-neoplastic renal findings. The haematoxylin-and-eosin–stained kidney sections prepared in the original study had faded and could not be evaluated; the paraffin-embedded blocks of 50 males from each group which had been stored for each observation period were sectioned and stained by haematoxylin and eosin for microscopic re-examination." The results from the re-examination did not change the renal carcinoma counts but removed two renal adenomas from the high exposure group resulting in no significant findings for adenomas (p_{Trend}=0.187) and a marginally significant finding for adenomas and carcinomas combined (p_{Trend}=0.062). The new pathology data does not negate the original pathology since they examined different slides and it cannot be established if the new slices are new tumors or cut through parts of the original tumors. The re-examination will not be used in this assessment since it ignores the previous findings.

JMPR (2017) only noted that there was a significant trend for malignant lymphomas in females but no significance for the pairwise comparison suggesting this was a reason to exclude this finding.

This study will be included in the overall evaluation of causation.

Table 15: Tumors of interest in male and female CD-1 mice from the 18-month feeding study of Takahashi (1999)

Tumor	Doses (mg/kg/day) or Tumor Incidence[1]				Trend Test p-value
Males	0	167.6	685	7470	
Kidney Adenomas	0/50	0/50	1/50	3/50	0.019 (0.333)[2]
Kidney Carcinomas	0/50	0/50	0/50	1/50	0.250 (---)[2]
Kidney Adenomas and Carcinomas	0/50	0/50	1/50	4/50	0.005 (0.333)[2]
Females	0	93.2	909	8690	
Malignant Lymphomas	3/50	1/50	4/50	6/50	0.050 (0.244)[2]

This is publication [52]. 1 – Doses are given in the rows marked "Males" and "Females", tumor counts appear on the rows with the individual tumors; * $0.01<p\leq0.05$ for Fisher's Exact Test; ** $p\leq0.01$ for Fisher's Exact Test; 2 – excluding the highest exposure group

Kumar (2001)[47] exposed Swiss Albino mice to glyphosate (>95% purity) in feed for two years. Fifty animals/group/sex were tested in four exposure groups (see Table 16).

The survival was decreased in the highest exposure group but this was not statistically significant and there was no other data indicating the MTD was exceeded for this study.

Kidney adenomas ($p_{Trend}=0.090$) in male mice demonstrated marginal statistical significance. No historical control data was available for kidney adenomas in Swiss Albino mice.

For the malignant lymphomas, **EFSA** described historical control data from the literature showing a mean response of 46/250=0.184 (18.4%) with a range of 6% to 30%; individual control data sets were not provided. There is a statistically significant increase in the highest exposure group $p_{Fisher}=0.038$ and a marginal trend in the response ($p_{Trend}=0.064$) in males. The responses at two of the doses are outside of the historical control range and the third dose is at the upper limit of the historical control range as presented by **EFSA**. However, this is a small historical control dataset for a tumor with a relatively high background tumor rate, thus placing too much emphasis on this historical control population is not warranted. Note that both the **EPA** and **EFSA** evaluations of the Kumar study had 10 animals with malignant lymphomas in controls whereas the original study data shows 12 [154].

This study also saw a marginally significant increase in malignant lymphomas in female mice ($p_{Trend}=0.070$). In these same groups there was a marginally statistically significant increase in enlarged mesenteric lymph nodes ($p_{Trend}=0.053$).

This study also saw a significant increase in hemangiomas in female mice (p_{Trend}=0.004); this increase was not mentioned by any of the regulatory agency documents reviewed. The increase was most dramatic in the mesenteric lymph nodes (p_{Trend}=0.016).

In summary, this study shows support for an increase for malignant lymphomas as a function of exposure in male and female Swiss Albino mice and an increase in hemangiomas in female Swiss albino mice. This study will be included in the overall evaluation of causation.

Table 16: Tumors of interest in male and female Swiss Albino mice from the 18-month feeding study of Kumar (2001) [100]

Tumor	Doses (mg/kg/day) or Tumor Incidence[1]				Trend Test p-value
Males	0	14.5	149.7	1453	
Kidney Adenomas	0/50	0/26	1/26	2/50	0.090
Kidney Carcinomas	0/50	0/26	0/26	0/50	----
Kidney Adenomas and Carcinomas	0/50	0/26	1/26	2/50	0.090
Malignant Lymphomas[2]	10/50	15/50	16/50	19/50*	0.064
Females	0	15	151.2	1466.8	
Malignant Lymphomas[2]	18/50	20/50	19/50	25/50	0.070
Hemangioma	1/50	0/50	0/50	5/50	0.004

1 – Doses are given in the rows marked "Males" and "Females", tumor counts appear on the rows with the individual tumors; 2 – tumor counts obtained from EFSA [148]; * 0.01<p≤0.05 for Fisher's Exact Test; ** p≤0.01 for Fisher's Exact Test

Pavkov and Turner (1987)[48] exposed CD-1 mice to glyphosate trimesium salt (56.2%) and 1% propylene glycol (wet weight vehicle) in feed for two years. Eighty animals/sex/group were tested in control, low- and mid-dose groups and 90 animals/sex were tested at the high dose. Exposure levels were 0, 11.7, 118 and 991 mg/kg/day in males and 0, 16, 159 and 1341 mg/kg/day in females. **EPA**[54] lists this study as completely negative for any cancer findings. No details on this study are provided by the EPA nor is it listed in the **Greim et al. (2015)** [58] manuscript. There was limited information on this study in a Data Evaluation Report from EPA (accession number 4021 40-06) that discussed findings from this study. EPA noted that body weight and food consumption were reduced in the highest exposure group, but the actual amounts of these reductions were not available. They also noted that the authors failed to make it clear that the tumors reported in the study had been histopathologically validated. Data was presented for tumors in the livers and lungs of male mice and the lungs of female mice. No other data is provided.

This study is not acceptable for inclusion in the evaluation of causation due to the lack of information on the tumor incidence in tissues other than liver and lung.

George et al. (2010)[45] exposed groups of 20 male Swiss Albino mice to a glyphosate formulation (Roundup Original, 36g/L glyphosate) at a dose of 25 mg/kg (glyphosate

equivalent dose) topically three times per week, topically once followed one week later by 12-o-tetradecanoylphorbol-13-acetate (TPA) three times per week, topically three times per week for three weeks followed one week later by TPA three times per week, or a single topical application of 7,12-dimethyl-benz[a]anthracene (DMBA) followed one week later by topical application of glyphosate three times per week for a total period of 32 weeks. Appropriate untreated, DMBA-treated, and TPA-treated controls were included. The group exposed to DMBA followed by glyphosate demonstrated a significant increase ($p<0.05$) in the number of animals with tumors (40% of the treated animals versus no tumors in the controls) indicating glyphosate has a promotional effect on carcinogenesis in the two-stage model in skin. This study addresses the question of whether glyphosate is more likely to cause skin tumors through initiation (starting the cancer process) or promotion (moving the process along after it starts). This study supports the overall concept that glyphosate can have an impact on tumor incidence.

EPA [140] discounted this study because it included only 20 animals per group, tested only males and did not conduct a histopathological analysis. It is hard to understand how EPA could reject a positive finding using 20 mice; typically one would ignore a negative study that had too few animals as not having sufficient statistical power to see an effect but never reject positive findings for this reason. Also, 20 animals per group is common for skin-painting initiation-promotion studies like the one presented here. Doing a study in only males is not a reason to ignore the positive findings in a study. Finally, in initiation-promotion studies of mouse skin, histopathological evaluation would be done if one were interested in separating papillomas from carcinomas. It is highly unlikely that the lesions seen in 40% of the DMBA/glyphosate treated mice were not papillomas or carcinomas.

Some members of the EPA SAP noted[121] that the rodent data were consistent with glyphosate acting as a tumor promoter but, because "*[t]here has been no direct test of this hypothesis (such as in a standard initiation-promotion bioassay)…,*" this "*conclusion was speculative.*" (page #). Because the EPA dismissed this study without any discussion, the SAP did not recognize there was an initiation-promotion supporting a promotional effect of glyphosate.

This study is included in the evaluation of causality as support for a promotional effect of glyphosate on some tumors.

7.1.5 Joint Analysis of Mouse Carcinogenicity Studies

In their evaluation of the mouse studies, **EPA**[54] and **EFSA**[55] chose to challenge the results in each study separately, dismiss the studies as showing no effect, and never compared results across the various studies. In response to the evaluation done by the **IARC**[6], **EFSA**[56] extracted the original data and did trend tests on kidney tumors, malignant lymphomas and hemangiosarcomas in male mice in five of the six mouse studies included in this evaluation. Rather than formally evaluate these cancer responses for consistency by pooling the data where appropriate, **EPA** and **EFSA** simply produced a table with the responses for each dose group in each study and concluded (subjectively) they were inconsistent. In addition, **EPA** and **EFSA** argued that doses

above 1000 mg/kg/day (there are only two of these) were outside the range of what would be tested today under OECD guidelines and should be excluded.

Table 17 summarizes the significance for all tumors of interest in CD-1 mice. For ease of discussion, we will refer to **Knezevich and Hogan (1983)** as study H, **Atkinson et al. (1993)** as study I, **Sugimoto (1997)** as study J, **Wood (2009)** as study K, and **Takahashi (1999)** as study L.

In CD-1 mice, there are five useful animal carcinogenicity studies and one study in Swiss Albino mice. As with the rats, consistency across studies can be addressed in two ways. The first is by simply looking at the overall findings to evaluate where they agree or disagree in terms of statistical significance. Table 17 summarizes the positive and negative findings for all cancers in which at least one study in CD-1 mice showed a significant trend. It is clear that not every tumor shows a positive trend with glyphosate exposure in every study.

A significant trend for kidney adenomas (p_{Trend}=0.019) and adenomas and carcinomas combined (p_{Trend}=0.005) was seen in male mice in study L, marginal trends were seen in study J (p_{Trend}=0.062 for both) and marginal trends were seen for carcinomas (p_{Trend}=0.063) and combined carcinomas and adenomas (p_{Trend}=0.065) for study H with no apparent increase in the remaining two studies. The significant findings in study L were no longer significant if the highest dose was removed because it possibly exceeded the maximum tolerated dose. Kidney tumors are rare in CD-1 mice and it would be appropriate to compare the marginal responses against historical controls. Using historical control data for kidney tumors from the EPA archives [145] on study H resulted in no significant association with adenomas (p_{Hist}=0.138) but significant increases in carcinomas (p_{Hist}=0.020) and adenomas and carcinomas combined (p_{Hist}=0.010) by Tarone's test. Using historical controls from 1990-1995 from the literature, resulted in a significant trend (p_{Hist}=0.005) for kidney adenomas in Study J. The pooled analysis of the data shows a significant trend for adenomas (p_{TrendA}=0.043), carcinomas (p_{TrendA}=0.009) and the combined tumors (p_{TrendA}=0.005)with no indication of different slopes between the studies. When the high dose animals in study L are removed, The adenomas (p_{TrendA}=0.038) and combined adenomas and carcinomas (p_{TrendA}=0.011) remain significant and the carcinomas become marginally significant (p_{TrendA}=0.077).

In male CD-1 mice, study H saw a significant increase in chronic interstitial nephritis (p_{Trend}=0.004) and a non-significant increase in thickening of the glomerular and/or tubular basal membranes (p_{Trend}=0.148) with a significant pairwise increase at the mid-dose (p_{Fisher}=0.036). Study I saw an increase in tubular dilatation (p_{Trend}=0.026) but no change in tubular hypertrophy (p_{Trend}=0.642) or focal tubular atrophy (p_{Trend}=0.248). Study J saw no change in tubular dilatation (p_{Trend}=0.913) but did see an increase in tubular atrophy (p_{Trend}=0.017) and tubular vacuolation (p_{Trend}=0.015). Study K showed no changes in vacuolation (p_{Trend}=0.830), dilatation (p_{Trend}=0.831), or chronic nephropathy (p_{Trend}=0.494). In Swiss albino mice, there were no non-neoplastic changes in the kidney. Data on kidney damage was not available for Study E.

Malignant lymphomas were significant in studies J (p_{Trend}=0.016) and K (p_{Trend}=0.007) and marginally significant in study I (p_{Trend}=0.087) in male mice. Malignant lymphomas are not rare in these mice so no historical control analysis was conducted. The pooled analysis was highly significant (p=0.003) but the studies were heterogeneous in slope because of the markedly different response in study H.

This is one of the most consistent positive findings in these studies in mice. Study I had a significant increase in thymus weight in the two highest exposure groups (p_{Fisher}<0.01 and p_{Fisher}<0.05 respectively – study author) in males and a non-significant (p not reported) increase in females. Studies I and J had a significant increase (trend test) in the number of males with enlarged mesenteric lymph nodes (p_{Trend}=0.024 and p_{Trend}=0.002 respectively). Study I had enlarged spleens (p_{Trend}=0.031) in males whereas J did not. Study J also had an increase in enlarged cervical lymph nodes (p_{Trend}=0.046) and other lymph nodes (p_{Trend}=0.047). Study H did not report macroscopic findings, study K had no enlarged lymphoreticular tissues and the data were not available from study E. Swiss albino mice showed a significant increase in the incidence of thymus enlargement in males (p_{Trend}=0.034).

Hemangiosarcomas were statistically significant in study I (p_{Trend}=0.004) and marginally significant in study J (p_{Trend}=0.062) in male mice. Hemangiosarcomas are very rare in 18-month animals with no tumors appearing in 26 historical control data sets and moderately rare (2.1%) in 24-month studies [152]. Using appropriate historical control data and Tarone's test results in a significant finding for studies I (p_{Hist}=0.001) and j (p_{Hist}=0.006). The pooled analysis was significant (p_{TrendA}=0.03) but the studies were heterogeneous in slope due entirely to study H.

Although there was a single positive finding in the lung in male mice with a significant increase in carcinomas in study K (p_{Trend}=0.028), all of the other analyses in the lung were not statistically significant including the pooled analyses.

In female mice, hemangiomas were significantly increased in study J (p_{Trend}=0.002) and the pooled analyses was also significant (p_{Trend}=0.031). Study J had a 10% response at the highest dose whereas the other studies had much lower response resulting in the positive pooled association.

Harderian gland adenomas were significantly increased in study J (p_{Trend}=0.04) and showed a non-significant increase in study K but were not significant for all of the remaining studies for adenomas, carcinomas and their combination. The pooled analyses failed to demonstrate a consistent increase.

There was a significant increase in adenomas and carcinomas combined in the lung for female mice in study H (p_{Trend}=0.016). None of the pooled analyses or any analyses in the remaining studies were significantly increased in the lung.

Finally, malignant lymphomas in female mice were significantly increased in study L (p_{Trend}=0.050) and marginally increased in study H (p_{Trend}=0.070). The increase seen in study L was not significant if the highest dose is removed because it may have exceeded the MTD. The remaining studies showed non-significant trends toward increasing risk

with increasing exposure and when combined, the five mice studies showed a significant increase in malignant lymphomas in female CD-1 mice (p_{TrendA}=0.012). Significance was reduced, but remained if the highest dose in study L was discarded (p_{TrendA}=0.050). Malignant lymphomas were significantly increased in Swiss albino mice (p_{Trend}=0.050).

There were no increases in enlargement of lymphoreticular tissues in female mice in studies I, J and K. Swiss albino mice showed a marginal increase in enlargement of mesenteric lymph nodes in females (p_{Trend}=0.053).

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Table 17: P-values for the Cochran-Armitage trend test and pooled logistic regression analysis for tumors with at least one significant trend test (p≤0.05) or Fisher's exact test (p≤0.05) in male and female CD-1 mice

Tumor	Individual study p-values for trend[1]					Common Trend	Heterogeneity Test
Males	H	I	J	K	L		
Kidney Adenomas	0.442 (0.138)[4]	0.938	0.062 (**0.009**)[4]	---[2]	**0.019**	**0.006**	0.268
Kidney Carcinomas	0.063 (**<0.001**)[4]	0.938	---[2]	---[2]	0.250	**0.031**	0.546
Kidney Adenomas and Carcinomas	0.065 (**0.008**)[4]	0.981	0.062 (**0.009**)[4]	---[2]	**0.005**	**<0.001**	0.106
Malignant Lymphomas	0.754	0.087	**0.016**	**0.007**	ND[3]	0.093	0.007
Hemangiosarcomas	0.505	**0.004**	0.062 (**0.005**)[4]	---[2]	ND[3]	**0.033**	0.007
Alveolar-Bronchiolar Adenomas	0.294	0.231	0.513	0.924	ND[3]	0.384	0.409
Alveolar-Bronchiolar Carcinomas	0.918	0.456	0.148	**0.028**	ND[3]	0.407	0.083
Alveolar-Bronchiolar Adenomas and Carcinomas	0.576	0.231	0.294	0.336	ND[3]	0.346	0.826
Females	H	I	J	K	L		
Hemangiomas	0.631	---[2]	**0.002**	0.438	ND[3]	**0.031**	0.155
Harderian Gland Adenomas	0.877	ND[3]	**0.040**	0.155	ND[3]	0.155	0.052
Harderian Gland Carcinomas	---[2]	ND[3]	---[2]	1.000	ND[3]	0.500	1.00
Harderian Gland Adenomas and Carcinomas	0.877	ND[3]	**0.040**	0.372	ND[3]	0.184	0.110
Alveolar-Bronchiolar Adenomas	0.999	0.144	0.800	0.656	ND[3]	0.996	0.211
Alveolar-Bronchiolar Carcinomas	0.183	0.110	0.623	0.601	ND[3]	0.268	0.544
Alveolar-Bronchiolar Adenomas and Carcinomas	0.985	**0.048**	0.842	0.688	ND[3]	0.982	0.241
Malignant Lymphomas	0.070[5]	0.484	0.294	0.353	**0.050**	**0.012**	0.995

[1] – Study H is Knezevich and Hogan (1983) [Table 11], Study I is Atkinson et al. (1993) [Table 12], Study J is Sugimoto (1997) [Table 13], Study K is Wood (2009) [Table 14], Study L is Takahashi (1999) [Table 15]; [2] – three dashes "---" indicates all tumor counts are zero; [3] – ND indicates there was no data available for this tumor in this study; [4] – using historical control data (see text for details) and Tarone's test; [5] – Spleen composite lymphosarcomas (malignant lymphomas) are also significantly increased in female mice in this study (see Table 11)

7.1.6 Discussion and Summary Animal Carcinogenicity Studies

As noted earlier, there has been a suggestion that using doses substantially larger than 1000 mg/kg/day exceeds the current limit dose set by the OECD. The only place in the **OECD guidance**[136] that addresses a dose of 1000 mg/kg/day is in paragraph 23 which reads:

> "*For the chronic toxicity phase of the study, a full study using three dose levels may not be considered necessary, if it can be anticipated that a test at one dose level, equivalent to at least 1000 mg/kg body weight/day, is unlikely to produce adverse effects. This should be based on information from preliminary studies and a consideration that toxicity would not be expected, based upon data from structurally related substances. A limit of 1000 mg/kg body weight/day may apply except when human exposure indicates the need for a higher dose level to be used.*"

This language does not preclude the use of a dose exceeding 1000 mg/kg/day nor does it advocate ignoring such doses when evaluating the results of an animal carcinogenicity study. In fact, the reasons for excluding a dose in an animal carcinogenicity study are clearly outlined in paragraph 90 within **OECD guidance**[28] and reads:

> "*If the main objective of the study is to identify a cancer hazard, there is broad acceptance that the top dose should ideally provide some signs of toxicity such as slight depression of body weight gain (not more than 10%), without causing e.g., tissue necrosis or metabolic saturation and without substantially altering normal life span due to effects other than tumours. Excessive toxicity at the top dose level (or any other dose level) may compromise the usefulness of the study and/or quality of data generated. Criteria that have evolved for the selection of an adequate top dose level include: (in particular) toxicokinetics; saturation of absorption; results of previous repeated dose toxicity studies; the MOA and the MTD.*"

While one study has a slight decrease in body-weight gain and another a more serious increase in mortality, there are only indications in one study of an exceedance in dose that would support ignoring the findings from any exposure group.

EPA[3] uses a slightly different criteria to determine which dose to include or exclude based on an earlier OECD document. These are spelled out in EPA's guideline document for carcinogenicity risk assessment[3]

> *"Other signs of treatment-related toxicity associated with an excessive high dose may include (a) significant reduction of body weight gain (e.g., greater than 10%), (b) significant increases in abnormal behavioral and clinical signs, (c) significant changes in hematology or clinical chemistry, (d) saturation of absorption and detoxification mechanisms, or (e) marked changes in organ weight, morphology, and histopathology. It should be noted that practical upper limits have been*

established to avoid the use of excessively high doses in long-term carcinogenicity studies of environmental chemicals (e.g., 5% of the test substance in the feed for dietary studies or 1 g/kg body weight for oral gavage studies [OECD, 1981]).” As before, this applies to only one study presented in this review.

Both of these guidelines make good scientific sense. In the 13 acceptable rodent carcinogenicity studies included in this evaluation, only one study had sufficient toxicity at the highest dose to justify removing the highest dose from the analysis. Hence, the analyses presented here did not drop the doses >1000 mg/kg/day. This is also supported by one member of the **EPA's SAP**[121].

Twenty-three chronic rodent carcinogenicity studies have been done using glyphosate as the test compound. Nine of these studies are unacceptable for use in an evaluation of causality leaving seven studies in rats, six studies in mice and one initiation/promotion study discussed later. Because of the large number of evaluations done in an individual animal carcinogenicity study, there is concern that the false-positive rates could be exaggerated. For example, if 20 evaluations are done and a finding is deemed significant if $p_{Trend}<0.05$, then you would expect that 20*0.05=1 evaluation would be positive simply due to chance.

Table 18 evaluates the probability that all of the observed tumor increases using the trend test are due to chance. The analysis is based upon the number of actual trend tests run in for the 13 studies discussed above. For each study, as discussed at the beginning of the section on animal carcinogenicity studies, trend tests were run for (a) any primary tumor for which there were at least three animals with a positive tumor finding when added together over all of the study groups, (b) when the tumor was a rare tumor and the use of historical controls via Tarone's historical control trend test was warranted or (c) when regulatory agencies provided a historical control populations and used it to argue that the observed trend was not important because it was in the range of the historical control data (in these cases, Tarone's test was also run with the data). The number of tests run for each sex/species/strain group are presented in Table 18 and is labeled as "Total Sites". The expected number of positive trend tests are calculated as Total Sites times p-value where the p-value being used is either 0.05 (Exp.<0.05) or 0.01 (Exp.<0.01). The number of actual observed trend tests with the associated P_{Trend} or P_{Hist} less than 0.05 or 0.01 are labeled as Obs.<0.05 and Obs.<0.01 respectively. In parentheses, next to the observed numbers, are the probabilities of seeing at least that observed number of positive trend tests using the binomial cumulative distribution function. For example, for male Sprague-Dawley rats, there were 101 total trend tests done for the 4 studies using these animals. At the 0.05 level, it is expected there would be 5.1 positive trend tests, 9 were observed with a probability of seeing this of 7% (0.07).

With the exception of female Sprague-Dawley rats and male albino mice, the observed number of tumors are greater than the expected number for the different sex/strain groups in rats (Table 18). For male Sprague-Dawley rats, 1 case with $p_{Trend}\leq0.01$ or $p_{Hist}\leq0.01$ are expected and two were observed (p=0.25). In female CD-1 mice and Swiss Albino mice, the expected and observed numbers are approximately equal except for

p<0.05 for female CD-1 mice where more were observed than expected. In male CD-1 mice, there were 3 tumors expected for $p_{Trend} \leq 0.05$ or $p_{Hist} \leq 0.05$ and 11 were observed (p<0.001) and there were 0.6 expected for $p_{Trend} \leq 0.01$ or $p_{Hist} \leq 0.01$ and 6 were observed (p<0.001). This clearly could not have occurred by chance alone. Even if one incorrectly groups all sexes and species together, there are 24.8 expected responses for $p_{Trend} \leq 0.05$ or $p_{Hist} \leq 0.05$ and 41 observed (p=0.001) and 4.4 expected responses for $p_{Trend} \leq 0.01$ or $p_{Hist} \leq 0.01$ and 19 observed (p<0.001). Thus, chance does not explain the positive results seen in these studies.

Table 18: Observed (Obs.) versus expected (Exp) tumor sites with significant trends in the 13 acceptable rodent carcinogenicity studies using glyphosate.

Species	Strain	Sex	Total Sites[1]	Exp. p<0.05	Obs. p<0.05 (prob.)[2]	Exp. p<0.01	Obs. p<0.01
Rat (7 studies)	Sprague-Dawley (4 studies)	M	125	6.3	9 (0.17)	1.3	4 (0.04)
		F	95	4.8	4 (0.52)	1.0	2 (0.25)
	Wistar (3 studies)	M	67	3.4	5 (0.24)	0.7	2 (0.15)
		F	58	2.9	4 (0.33)	0.6	1 (0.44)
Mouse (5 studies)	CD-1 (5 studies)	M	60	3.0	11 (<0.001)	0.6	8 (<0.001)
		F	63	3.2	6 (0.09)	0.6	1 (0.47)
	Albino (1 study)	M	24	0.7	0 (1)	0.1	0 (1)
		F	14	0.7	1 (0.51)	0.1	1 (0.13)
Rats (7 studies)	All (7 studies)	M	192	9.6	14 (0.10)	1.9	6 (0.013)
		F	153	7.7	9 (0.36)	1.5	3 (0.20)
		Both	345	17.3	23 (0.02)	3.5	9 (0.01)
Mice (5 studies)	All (5 studies)	M	74	3.7	11 (0.001)	0.7	8 (<0.001)
		F	77	3.9	7 (0.09)	0.8	2 (0.18)
		Both	151	7.6	18 (0.001)	1.5	10 (<0.001)
All (12 studies)	All (12 studies)	M	266	13.3	25 (0.002)	2.7	14 (<0.001)
		F	230	11.5	16 (0.12)	2.3	5 (0.08)
		Both	496	24.8	41 (0.001)	5.0	19 (<0.001)

1 – number of trend tests actually conducted; 2 – probability of seeing the number of observed significant findings or more

There are numerous studies in the literature that relate to the cancer findings shown in Tables 3-5. Some of the studies are done using pure glyphosate, but many use a glyphosate-based herbicide (GBH) and present the results in glyphosate-equivalent doses. GBHs contain adjuvants, some of which are also likely to be highly toxic. In what follows, these related studies are discussed and care is taken to note whether the exposure is to glyphosate or a GBH. Caution should be used in interpreting the results using the GBHs since, in most cases, it is not clear if the resulting toxicity is due to the glyphosate in the GBH or the adjuvant(s).

Increases in kidney adenomas and carcinomas (combined) are seen in male CD-1 mice and increases in adenomas are seen in Swiss albino mice and SD rats in the reanalysis in this review. A number of short-term toxicity studies have demonstrated damage to the kidneys in laboratory animals from exposure to glyphosate or GBHs. Turkman et al.

[155] saw significant ($p<0.05$) increases in malondialdehyde (MDA) levels and decreases in glutathione (GSH) levels in male Wistar albino rats exposed to the GBH Knockdown 48SL. They also saw degeneration in the tubulur epithelial cells and expansion and vacuolar degeneration in glomerulus Bowman's capsule ($p<0.05$ for both). Dedeke et al. [156] also saw significant changes in MDA, GSH and several other kidney biomarkers from exposure to the GBH Roundup in male albino rats. They also studied glyphosate alone in equal doses to the GBH and saw smaller, but still significant increases in MDA and GSH, but not in the other biomarkers. In addition, they found that the amount of glyphosate in kidney tissue was substantially higher from exposure to the GBH than from exposure to glyphosate alone. Tang et al. [157] saw proximal and distal tubular necrosis ($p<0.01$), glomerular toxicity ($p<0.01$) and a reduction in weight ($p<0.05$) in the kidneys of male SD rats exposed to glyphosate. They used a histopathological score and saw significant changes ($p<0.01$) even down to a dose of 5 mg/kg body weight. Hamdaoui et al. [158] saw numerous histological changes and changes in urine and plasma associated with renal disfunction in female Wistar rats exposed to the GBH Kalach 360 SL. Kidney damage included fragmented glomeruli, necrotic epithelial cells, and tubular dilatation, inflammation, proximal tubular necrosis and distal tubular necrosis. Tizhe et al. [159] also saw glomerular degeneration, mononuclear cell infiltration and tubular necrosis in male and female Wistar rats exposed to the GBH Bushfire. Cavusoglu et al. [160] saw similar changes in blood chemistry and kidney pathology in male albino mice exposed to the GBH Roundup Ultra-Max. Wang et al. (2019) [85] saw kidney damage to tubular cells in Vk*MYC mice exposed to glyphosate in water.

In humans, GBHs are suspected to be involved in chronic kidney disease of unknown etiology (CKDu) in Sri Lanka, Mexico, Nicaragua, El Salvador and India [161-163]. Finally, the English abstract of a Chinese article by Zhang et al. [164] describe significant increases ($p<0.05$) in abnormal hepatorenal function in workers occupationally exposed to glyphosate from 5 glyphosate-producing factories.

Dose-related increases in malignant lymphomas are seen in male and female CD-1 mice and marginal increases are seen in male and female Swiss albino mice in the reanalysis presented here. Wang et al. (2019) [85] exposed male and female Vk*MYC mice from the C57Bl/6 genetic background to glyphosate (purity not provided) at an exposure of 1 g/L in drinking water for 72 weeks (approximately 18 months) with an appropriate control. In addition, using the same mice, 7-day exposures were given at doses of 0, 1, 5, 10 and 30 g/L of glyphosate ($n=5$ per group). Glyphosate induced splenomegaly in both wild type (WT) and Vk*MYC mice. Both WT and Vk*MYC mice demonstrated a significant increase ($p<0.05$) in IgG levels when compared to controls. Vk*MYC treated mice had a clear M-spike (an indicators of multiple myeloma - MM), WT mice had a weaker M-spike and no M-spike was detected in untreated animals regardless of genetics. In addition, there were multiple hematological abnormalities in treated versus untreated mice that were consistent with MM. Activation-induced cytidine deaminase (AID, a marker of *monoclonal gammopathy of undetermined significance* induction, a

precursor of MM) was upregulated in both bone marrow and spleen of both Vk*MYC and WT mice in the 72-week study. The same upregulation in the spleen and bone marrow were seen in the 7-day exposure animals in a dose-dependent fashion. A smaller dose-dependent increase was seen in lymph nodes. This upregulation of AID supports an AID-mediated mutational mechanism for the induction of MM and malignant lymphoma in these mice.

In humans, GBHs have been shown to increase the risk ratios for non-Hodgkins lymphomas (NHL) in several meta-analyses [5, 7, 26, 27]. For over 30 years, mouse models have been studied and evaluated as surrogates for NHL [80-84]. Classification systems for humans and mice indicate a strong similarity between malignant lymphomas in mice and NHL in humans.

Skin keratoacanthomas are increased by glyphosate in male SD rats and male Wistar rats. Skin basal-cell tumors are also increased in male SD rats in the reanalysis in this review. George et al. (2010) [45] exposed Swiss Albino mice to a glyphosate formulation (Roundup Original, 36g/L glyphosate) in a typical skin-painting initiation-promotion study using 12-o-tetradecanoylphorbol-13-acetate (TPA) as a promoter and 7,12-dimethyl-benz[a]anthracene (DMBA) as an initiator. The group exposed to DMBA followed by glyphosate demonstrated a significant increase ($p<0.05$) in the number of animals with tumors (40% of the treated animals versus no tumors in the controls) indicating the GBH has a promotional effect on carcinogenesis in the two-stage model in skin. Several in-vitro studies using human skin cells [165-167] have shown an increase in oxidative stress following exposure to glyphosate.

This review shows hepatocellular adenomas are increased by exposure to glyphosate in male SD rats and Wistar rats. Glyphosate has been shown to affect energy metabolism of mitochondria [168-171] and AST, ALT, and LDH [172] but not peroxisome proliferation or hypolipidemia [173] in the livers of Wistar rats. Transcriptome analyses of liver tissue in Sprague-Dwaley rats chronically exposed to the GBH Roundup Grand Travaux Plus suggest liver tissue damage is occurring [174]. Glyphosate and GBHs also seem to induce oxidative stress in the livers of several rat strains [157, 175, 176].

Adrenal cortical carcinomas are increased in female Sprague-Dawley rats in the reanalysis in this review. There is also a suggestion of an increase in adrenal pheochromocytomas in male Wistar rats and of pituitary adenomas in male and female Wistar rats. Owagboriaye et al. (2019) [177] saw a significant increase in adrenal hormones aldostererone and corticosterone in a dose-dependent fashion following exposure to a GBH (Roundup Original) in male albino rats but not following exposure to equivalent doses of glyphosate (purity not given). Significant changes in adrenocorticotropic hormone were also seen for the GBH but not glyphosate. In contrast, Pandey and Rudraiah (2015) [178] saw a significant reduction in adreno-corticotropic hormone levels at similar doses in Wistar rats. Romano et al. (2010) saw a reduction in adrenal weights from exposure to the GBH Roundup Transorb in newly-weaned male Wistar rats but saw no differences in corticosterone levels except a rather large, non-statistical increase at the lowest exposure group. Changes in these and other hormones in these three papers suggest GBHs could have an impact on the

hypothalamic-pituitary-adrenal axis that, after lifetime exposure, could induce cancers in the adrenal cortex and/or pituitary.

This reanalysis shows an inconsistent effect of glyphosate on the rates of mammary gland adenomas, carcinomas and combined adenomas and carcinomas in female Wistar rats but not in SD rats. Seralini et al. (2014) [39] saw an increase in mammary tumors in female SD rats exposed to the GBH GT Plus with associated hypertrophies and hyperplasia. Glyphosate and GBHs have also been shown to disrupt estrogen receptor alpha in rats [179] and to alter cellular replication and genotoxicity in estrogen-sensitive cell lines [180-186].

The longest study in male Sprague-Dawley rats showed an increase in testicular interstitial cell tumors after reanalysis. Several studies have seen changes in aromatase, testosterone and/or estrogen levels in male rats exposed to glyphosate or GBHs [184, 187-193].

The reanalysis in this review show an inconsistent increase in thyroid C-cell adenomas and/or carcinomas in male and female SD rats and thyroid follicular cell adenomas in male SD rats. De Souza et al. (2017) [194] exposed male Wistar rats to the GBH Roundup Transorb from gestational day18 to postnatal day 5 and examined the animals for thyroid hormone effects at postnatal day 90. They saw dose-dependent decreases in thyroid stimulating hormone but no changes in circulating triiodothyronine or thyroxine. Genomic analysis suggested that genes involved in thyroid hormone metabolism and transport were probably involved in these alterations. In humans, Samsel et al. (2013) [195] hypothesized that glyphosate intake could interfere with selenium uptake, impacting thyroid hormone synthesis and increasing thyroid cancer risks. Using data from the Agricultural Health Study, Shrestha et al. (2018) [196] saw an association between ever/never use by farmworkers of GBHs and hypothyroidism (OR=1.28, 95% CI 1.07-1.52) and for the two lowest categories of intensity of use, but not the highest category.

7.1.7 Conclusion for Animal Carcinogenicity Studies

There are several general issues that pertain to all animal carcinogenicity studies. There is considerable genetic variability across animal strains both over time and space. It is difficult to compare experiments done in different laboratories even when using the same strain of animal. This is obvious when you examine the rates for hepatocellular adenomas in Wistar rats across the three studies using this strain. Thus, each study should be considered separately with regard to the findings in that study before being compared across studies.

The use of a p-value of 0.05 as the cut off for increasing tumor incidence does not account for trends in the data across multiple studies. Three studies with marginal responses of 6-8% in a given tumor could, when pooled for analysis, lead to highly significant findings. This issue is well-recognized in epidemiology but not usually considered in toxicology because of a lack of replicate studies. This case is fairly unique because of the larger number of studies available for analysis and requires a more

rigorous evaluation of the data such as the pooled analysis presented in this report.

Pooling of the data for the evaluation of replicate studies makes sense as it addresses the question "Does the data as a whole support a finding of increased cancer incidence in these studies?" Some toxicologists may argue that the studies are not replicates and hence cannot be pooled. But if they are not replicates, then they cannot be compared to see if there is consistency across the studies. This is because there may be some subtle change from one study to another that leads to a positive finding in one study but a negative finding in other studies. By using study and duration of exposure (as an ordinal variate crossed with dose) as variables in the pooled analysis, we allow for differences between the studies to account for this concern. Thus, either the studies are not good replicates and you cannot compare across studies and you cannot pool them, or they are good replicates so you can compare across studies and you can pool them. There is no argument that would support a comparison across studies that is appropriate when pooling is inappropriate.

There were seven rat studies and six mouse studies that were of sufficient quality and with sufficient details available for inclusion in this evaluation. Table 19 summarizes the findings of the animal studies by sex/species/strain.

Glyphosate has been demonstrated to cause cancer in two strains of rats and two strains of mice. There is clear evidence that glyphosate causes kidney adenomas, skin keratoacanthomas and skin basal cell tumors in male Sprague-Dawley rats and adrenal cortical carcinomas in female Sprague-Dawley rats. There is clear evidence that glyphosate causes hepatocellular adenomas and skin keratocanthomas in male Wistar rats. There is clear evidence that glyphosate causes hemangiosarcomas, kidney tumors and malignant lymphomas in male CD-1 mice and hemangiomas and malignant lymphomas in female CD-1 mice. There is clear evidence that glyphosate causes hemangiomas in female Swiss albino mice. There is some evidence that glyphosate causes testicular interstitial cell tumors, pancreas islet-cell adenomas and carcinomas, hepatocellular adenomas in male Sprague-Dawley rats. There is some evidence that glyphosate causes pituitary adenomas in male and female Wistar rats and mammary gland adenomas and carcinomas in female Wistar rats. There is some evidence that glyphosate causes malignant lymphomas in male and female and kidney tumors in male Swiss albino mice. There is equivocal evidence that glyphosate causes thyroid c-cell adenomas and carcinomas in male and female Sprague-Dawley rats, and thyroid follicular cell adenomas and carcinomas in male rats. There is equivocal evidence glyphosate causes adrenal pheochromocytomas in male Wistar rats. There is equivocal evidence glyphosate causes kidney adenomas in male Swiss albino mice.

Thus, glyphosate causes cancer in mammals.

Table 19: Summary of level of evidence1 for tumors observed to have a significant trend in 13 rodent carcinogenicity studies in male and female, mice and rats.

Tumor	Males				Females			
	SD Rat	Wistar Rat	CD-1 Mouse	Swiss Mouse	SD Rat	Wistar Rat	CD-1 Mouse	Swiss albino mouse
Adrenal cortical carcinoma					CE			
Alviolar-Bronchiolar tumor			NE				NE	
Harderian gland tumor							NE	
Hemangioma							CE	CE
Hemangiosarcomas			CE					
Kidney tumor	CE		CE	SE				EE
Liver Adenoma	SE	CE						
Mammary tumor						SE		
Malignant lymphoma			CE	SE			CE	
Pancreas Islet Cell Tumor	SE							
Pituitary tumor		SE				SE		
Skin basal-cell tumor	CE							
Skin keratoacanthoma	CE	CE						
Thyroid C-cell tumor	EE				EE			
Thyroid follicular-cell tumor	EE							
Testis interstitial-cell Tumor	SE							

1 – criteria as defined in Section 7.1.1: CE=clear evidence; SE=some evidence; EE=equivocal evidence; NE=no evidence

7.2 Mechanisms Relating to Carcinogenicity

Many human carcinogens act via a variety of mechanisms causing various biological changes, taking cells through multiple stages from functioning normally to becoming invasive with little or no growth control (carcinogenic). **Hanahan and Weinberg (2011)**[197] identified morphological changes in cells as they progress though this multistage process and correlated these with genetic alterations to develop what they refer to as the "hallmarks of cancer." These hallmarks deal with the entire process of carcinogenesis and not necessarily with the reasons that cells begin this process or the early stages in the process where normal protective systems within the cells remove potentially cancerous cells from the body. While tumors that arise from a chemical insult to the cell may be distinct from other tumors by mutational analysis, they all

exhibit the hallmarks as described by **Hanahan and Weinberg (2011)**.

Systematic review of all data on the mechanisms by which a chemical causes cancer is complicated by the absence of widely accepted methods for evaluating mechanistic data to arrive at an objective conclusion on human hazards associated with carcinogenesis. Such systematic methods exist in other contexts[198], but are only now being accepted as a means of evaluating literature in toxicological evaluations[2, 199-202].

In this portion of the report, I am focusing on the mechanisms that can cause cancer. **Smith et al. (2015)**[75] discussed the use of systematic review methods in identifying and using key information from the literature to characterize the mechanisms by which a chemical causes cancer. They identified 10 "Key Characteristics of Cancer" useful in facilitating a systematic and uniform approach to evaluating mechanistic data relevant to carcinogens. These 10 characteristics are presented in Table 20 (copied from Table 1 of **Smith et al. (2015)**[75]). While there is limited evidence on glyphosate for most of the key characteristics, genotoxicity (characteristic two) and oxidative stress (characteristic five) have sufficient evidence to warrant a full review.

7.2.1 Genotoxicity

Genotoxicity refers to the ability of an agent (chemical or otherwise) to damage the genetic material within a cell, thus increasing the risks for a mutation. Genotoxic substances interact with the genetic material, including DNA sequence and structure, to damage cells. DNA damage can occur in several different ways, including single- and double-strand breaks, cross-links between DNA bases and proteins, formation of micronuclei and chemical additions to the DNA.

Just because a chemical can damage DNA does not mean it will cause mutations. So, while all chemicals that cause mutations are genotoxic, all genotoxic chemicals are not necessarily mutagens. Does that mean that the genotoxicity of a chemical can be ignored if all assays used for identifying mutations in cells following exposure to a chemical are negative? The answer to that question is no and is tied to the limitations in tests for mutagenicity (the ability of a chemical to cause mutations in a cell). It is unusual to see an evaluation of the sequence of the entire genome before exposure with the same sequence after exposure to determine if the genome has been altered (mutation). There are assays that can evaluate a critical set of genes that have previously been associated with cancer outcomes (e.g. cancer oncogenes), but these are seldom applied. In general, mutagenicity tests are limited in the numbers of genes they actually screen and the manner in which these screens work.

Because screening for mutagenicity is limited in scope, any genetic damage caused by chemicals should raise concerns because of the possibility of a mutation arising from that genetic damage. In what follows, I will systematically review the scientific findings available for evaluating the genotoxic potential of glyphosate. This will be divided into six separate sources of data based on the biological source of that data: (1) data from exposed humans, (2) data from exposed human cells in a laboratory setting, (3) data from exposed mammals (non-human), (4) data from exposed cells of mammals (non-

human) in the laboratory, (5) data from non-mammalian animals and others, and (5) data from cells from non-mammalian animals and others. These six areas are based upon the priorities one would apply to the data in terms of impacts. Seeing genotoxicity in humans is more important than seeing genotoxicity in other mammals, which is more important than seeing genotoxicity in non-mammalian systems. In addition, seeing genotoxicity in whole, living organisms (*in vivo*) carries greater weight than seeing responses in cells in the laboratory (*in vitro*). Basically, the closer the findings are to real, living human beings, the more weight they should be given.

Table 20: Key characteristics of carcinogens, Smith et al. (2016)[65]

Characteristic	Examples of relevant evidence
1. Is electrophilic or can be metabolically activated	Parent compound or metabolite with an electrophilic structure (e.g., epoxide, quinone), formation of DNA and protein adducts
2. Is genotoxic	DNA damage (DNA strand breaks, DNA–protein cross-links, unscheduled DNA synthesis), intercalation, gene mutations, cytogenetic changes (e.g., chromosome aberrations, micronuclei)
3. Alters DNA repair or causes genomic instability	Alterations of DNA replication or repair (e.g., topoisomerase II, base-excision or double-strand break repair)
4. Induces epigenetic alterations	DNA methylation, histone modification, microRNA expression
5. Induces oxidative stress	Oxygen radicals, oxidative stress, oxidative damage to macromolecules (e.g., DNA, lipids)
6. Induces chronic inflammation	Elevated white blood cells, myeloperoxidase activity, altered cytokine and/or chemokine production
7. Is immunosuppressive	Decreased immunosurveillance, immune system dysfunction
8. Modulates receptor-mediated effects	Receptor in/activation (e.g., ER, PPAR, AhR) or modulation of endogenous ligands (including hormones)
9. Causes immortalization	Inhibition of senescence, cell transformation
10. Alters cell proliferation, cell death or nutrient supply	Increased proliferation, decreased apoptosis, changes in growth factors, energetics and signaling pathways related to cellular replication or cell cycle control, angiogenesis

Abbreviations: AhR, aryl hydrocarbon receptor; ER, estrogen receptor; PPAR, peroxisome proliferator–activated receptor. Any of the 10 characteristics in this table could interact with any other (e.g., oxidative stress, DNA damage, and chronic inflammation), which when combined provides stronger evidence for a cancer mechanism than would oxidative stress alone.

The data being included in this review come from the peer-reviewed scientific literature, the summaries of reports in regulatory documents that are proprietary and for which I have limited access to the original work, and reports from industry that are proprietary to which I have been given greater access. All of these studies are included in the overall evaluation of causation.

7.2.1.1 Genotoxicity in Humans in-vivo

Three studies have evaluated the potential genotoxicity of glyphosate formulations in exposed humans. **Paz-y-Miño et al. (2007)**[203] analyzed the blood of 24 exposed individuals (living within 3 kilometers of spraying) and 21 unexposed individuals (living 80 kilometers away from the spraying area) for DNA damage using the comet assay. All study subjects were from Ecuador and none of the controls or exposed individuals smoked, drank alcohol, took non-prescription drugs or had been exposed to pesticides during the course of their normal daily lives. Exposed and control individuals did some cultivating and harvesting but without pesticides or herbicides. Exposed individuals were analyzed within two months of spraying for the eradication of plants associated with illegal narcotics. An average of 200 cells per person were ranked between 0-400 depending on the amount of DNA in the comet's tail in order to calculate the mean amount of DNA damage. There was a significant difference between the mean total migration level of exposed individuals to controls ($p<0.001$). Data was given for each individual classified into five groups based upon the amount of DNA in the comet's tail. There was clearly a shift in the distribution of DNA in cells with the controls never seeing scores in the top two categories while all but three exposed had some scores in the top two categories. In essence, some of the DNA had been fragmented by the exposure.

In a second study by the same group, **Paz-y-Miño et al. (2011)**[204] evaluated the karyotypes (the chromosome count of the individuals and any alterations to the chromosomes as seen under a microscope) of 92 people living in 10 communities in northern Ecuador. Controls were from areas without spraying and both controls and exposed subjects had no history of exposure to smoking or other genotoxic compounds. This study saw no changes between controls and exposed subjects for 182 karyotypes evaluated.

Bolognesi et al. (2009)[62] studied women of reproductive age and their spouses in five areas of Colombia, four of which are subject to spraying for either narcotics control or sugar cane growing. There were 60 subjects from the Santa Marta area (organic coffee is grown without the use of pesticides), 52 from Boyaca (manual spraying for illicit drugs), 58 from Putumayo (aerial spraying for illicit drugs using a glyphosate formulation), 63 from Nariño (same exposure as Putumayo) and 28 from Valle del Cauca (aerial spraying of Roundup 747 (74.7% glyphosate) without additional adjuvant for sugar cane maturation). All subjects were interviewed with a standardized questionnaire designed to obtain information about current health status, health history, lifestyle and potential exposure to possible confounding factors (smoking, use of medicinal products, severe infections or viral diseases during the last six months, recent vaccinations, presence of known indoor/outdoor pollutants, exposure to diagnostic x-rays, and previous radio- or chemotherapy). In Santa Marta, blood samples were taken

once, during the initial interview. In Boyaca, blood samples were taken at the initial interview and 1 month later. In Nariño, Putumayo and Valle del Cauca, blood samples were taken at the initial interview, within five days after spraying and 4 months later. In lymphocytes, binucleated cells with micronuclei (BNMN) were lowest in Santa Marta and similar in the four exposed regions prior to exposure. Statistically significant increases in BMNM in Nariño, Putumayo and Valle del Cauca were seen between first and second sampling. The mean BNMN in Nariño and Putumayo was greater in respondents who self-reported direct contact with sprayed fields, but differences were not statistically significant. Multiple linear regression demonstrated statistically significant increases in BMNM in all four exposed regions post exposure when compared to pre-exposure and controlling for all other variables ($p<0.001$). The largest total change in mean BMNM values pre-exposure compared to immediate post exposure occurred in Valle del Cauca where spraying is done using Roundup with no additional adjuvant.

Kier (2015)[205] identified 16 additional studies of pesticide use that included some exposure to glyphosate. Eleven of the 16 studies demonstrated some degree of genotoxicity in the human populations studied but did not adequately attribute the exposure primarily to glyphosate so they are not included in this review. **Benedetti et al. (2016)** [206] also demonstrated an increase in DNA damage in humans exposed to pesticides but did not attempt to determine which pesticides were causing the DNA damage.

In summary, two of the three studies in which genotoxicity endpoints were evaluated in humans in areas with exposure to glyphosate spraying showed statistically increased changes in DNA damage in blood. In the strongest study, in three areas where chromosomal damage (micronuclei) was examined in individuals pre- and post-spraying (<5 days) showed statistically significant increases. In one other area where post-exposure damage was measured one month after exposure, there was little change.

7.2.1.2 Genotoxicity in Human Cells (in vitro)

Studies have explored the *in vitro* genotoxicity of glyphosate using a variety of different cell types (lymphocytes, fibroblasts, and immortalized cells from cancers of the larynx, mouth, blood and liver) using several different assays for markers of genotoxicity with or without metabolic activation.

Mladinic et al. (2009)[207] induced DNA strand breaks (comet assay) from exposure to glyphosate (purity not given) in lymphocytes from three healthy human donors (questionnaire used to exclude genotoxic exposures) at concentrations of 3.5, 92.8 and 580 µg/ml with S9 activation and saw effects at only the highest doses for cells without S9 activation.

Alvarez-Moya et al. (2014)[208] conducted a similar study using lymphocytes from human volunteers (questionnaire used to exclude genotoxic exposures) and exposure to glyphosate (96% purity) at concentrations of 0.12, 1.2, 12 and 120 µg/ml. A significant increase in DNA strand breaks (comet assay) was seen for all exposure groups with a clear dose-response relationship without metabolic activation (metabolic activation was

not tested).

Using human HEP-2 cells, **Manas et al. (2009)**[209] induced DNA damage (comet assay) by glyphosate (96% pure) at all concentrations ranging from 676 µg/ml to 1270 µg/ml (no S9 activation tested). Cell viability at the highest concentration was below 80% and values at the other concentrations were not given.

Monroy et al. (2005)[210] induced significant DNA damage (comet assay) in fibroblast GM 38 cells at concentrations of glyphosate (technical grade, purity not given) ranging from 676 µg/ml to 1000 µg/ml with a clear dose-response pattern. Over this same concentration range, they also saw concentration-dependent decreases in cell viability at all doses making the comet assay results difficult to interpret. In a similar analysis in the same paper, using fibrosarcoma HT1080 cells, they also saw concentration-dependent DNA damage and loss of cell viability. Activation by S9 was not used in either experiment.

Lueken et al. (2004)[211] induced DNA damage (comet assay) in fibroblasts GM 5757 at a concentration of glyphosate (98.4% purity) of 12,680 µg/ml in combination with exposure to 40 or 50 mM H_2O_2. Activation by S9 was not used in this experiment. According to the authors, cell viability at this exposure level was above 80%.

Koller et al. (2012)[212] significantly induced DNA damage (comet assay) in human TR146 cells (buccal carcinoma cells) from exposure to glyphosate (>95% purity) in a dose-dependent fashion at concentrations of 20 and 40 µg/ml. Above 40 µg/ml, there was a significant increase in tail intensity relative to controls, but the actual amount increased did not change as the dose increased (plateau). Using Roundup (Ultra Max) the authors saw virtually the same level of DNA damage at 20 and 40 µg/ml, but the concentration response continued to increase above that exposure. These experiments did not use S9 activation. They also used the CBMN assay in the same system to evaluate the total number of micronuclei in binucleated cells (MNI), the number of binucleated cells with micronuclei (BN-MNI), the number of nuclear buds (NB) and the number of nucleoplasmic bridges (NPB) caused by glyphosate and Roundup exposure. Two endpoints (NB, NPB) had significant increases at concentrations of 10, 15 and 20 µg/ml and two (MNi, BN-MNi) were significantly elevated for concentrations of 15 and 20 µg/ml. Equivalent Roundup exposures resulted in significant increases in all four measures of DNA damage at 10, 15 and 20 µg/ml. The results for the Roundup were greater than for glyphosate alone.

Gasnier at al. (2009)[181] exposed cells from the hepatoma cell line HepG2 to glyphosate (purity not given) and four glyphosate formulations. Only one glyphosate formulation was tested for DNA damage (comet assay) and they saw significant effects at equivalent concentrations of 0.05 µ/ml to 4 µg/ml of glyphosate (p-values not given). No p-values are provided and presentation of the results does not provide a clear means to compare these results with other studies. This study will not be used in the evaluation.

Manas et al. (2009)[209] obtained human blood samples from three healthy, non-smoking women and three healthy men with no history of pesticide exposure.

Lymphocytes were cultured with glyphosate (96% purity) at concentrations of 34, 203, and 1015 µg/ml with no statistically significant changes in chromatid breaks, chromosome breaks, chromatid gaps, chromosome gaps, dicentrics, acentric fragments, or endoreduplication.

Mladinic et al. (2009)[213] used blood from three non-smoking, healthy volunteers to evaluate the formation of micronuclei, nuclear buds and nucleoplasmic bridges as a function of exposure to glyphosate (98% purity). Significant changes in micronuclei were seen following exposure to glyphosate at 92.8 and 580 µg/ml in S9 activated cells, but not those without metabolic activation. Changes in nuclear buds were seen at 580 µg/ml for both S9 activated and non-activated cells while significant changes in nucleoplasmic bridges were seen only at 580 µg/ml in S9 activated cells. This study contained a positive control (ethyl methanesulfonate at 200 µg/ml) which was also negative in all assays, many times showing effects below that seen for glyphosate.

Bolognesi et al. (1997)[214] obtained blood from two healthy female donors and exposed it to glyphosate (99.9% purity) or a Roundup formulation (30.4% glyphosate). At concentrations of 1000, 3000 and 6000 µg/ml of glyphosate and at 100 and 330 µg/ml of glyphosate formulation, significant changes in sister chromatid exchanges (SCEs) were seen. At 330 µg/ml, a non-significant increase in SCEs was seen for glyphosate alone that was approximately 20% below that seen for an equivalent glyphosate exposure from the Roundup formulation. This study did not consider S9 activation.

Lioi et al. (1998)[209, 215] obtained blood from three healthy donors and exposed it to glyphosate (>98% purity). At concentrations of 1.4, 2.9, and 8.7 µg/ml of glyphosate, significant changes in sister chromatid exchanges (SCEs) and chromosomal aberrations were seen. This study did not consider S9 activation.

Vigfusson and Vyse (1980)[216] exposed cultured human lymphocytes from two people to Roundup (% glyphosate unknown) at concentrations of 250, 2500 and 25000 µg/ml. Results for the highest concentration were not provided due to lack of cell growth in culture. SCEs were shown to be significantly increased for the remaining two concentrations in one donor and only for the lowest concentration in the other. While the relative SCE counts seen in this paper are similar to those from **Bolognesi et al. (1997),** the absolute counts in the controls are roughly three times higher in this study. This study did not consider S9 activation.

Kim et al. (2017) [217] exposed human peripheral blood lymphocytes from 9 males and six females aged 20 to 24 to Roundup Ultramax (570 grams glyphosate per liter) at concentrations of 300 ng/mL of glyphosate with and without melatonin for 24 hours. Sister chromatid exchanges (SCEs) were significantly increased in all glyphosate formulation groups compared to control, but less so in the groups with melatonin as well as the glyphosate formulation.

Kasuba et al. (2017) [218] exposed human HepG2 cells in-vivo to glyphosate (analytical grade) glyphosate for 4 hours and 24 hours at doses of 0.5 µg/mL, 2.91 µg/mL and 3.5 µg/mL. There were no differences in cellular proliferation between the exposed cells

and the controls. There were significant decreases in tail intensity (comet assay) between the treated groups and control after 4 hours of exposure but not after 24 hours of exposure. After 4 hours of exposure, there was a significant increase in micronuclei in binucleated cells at all three exposure groups and a significant decrease in all exposed groups after 24 hours. Similarly, nuclear buds in binucleated cells were increased in all exposure groups following 4 hours of exposure and decreased after 24 hours of exposure.

Kwiatkowska et al. (2017) [219] exposed human peripheral blood mononuclear cells obtained from 9 healthy volunteers to glyphosate (purity 95%) for 24 hours at six different concentrations ranging from 0.1 to 10 mM. DNA damage (comet assay) was significantly increased in the highest 4 exposures. DNA repair was assessed after 120 minutes (difference in tail intensity in the comet assay) and showed that DNA damage was effectively repaired, but not completely by this time.

Luo et al. (2017) [220] exposed human L-02 hepatocytes Roundup (41% glyphosate) at doses of 60, 80, 120, 150 and 180 mg/L for 24 hours. DNA damage was examined using the Apoptotic DNA Ladder (a marker of apoptosis that can be produced by DNA damage). They saw a dose-dependent drop in survival across all exposures and a dose-dependent increase in DNA fragmentation.

Townsend et al. (2017) [221] human Raji cells (Burkitt's Lymphoma cells) to glyphosate (95% purity) for up to 2 hours at concentrations (7) ranging from 1 µM to 15mM. Glyphosate was cytotoxic to cells at 10 and 15 mM. DNA damage (comet assay) was significantly increased at 1 and 5 mM with peaks after 80 and 60 minutes of exposure respectively. For both doses, DNA damage reduced after these peaks. Cells treated a second time 1 hour after initial exposure did not show the same drop as seen from the original exposure. The remaining exposures showed no increase in DNA damage.

Anifandis et al. (2018) [222] fresh semen samples from 30 healthy male volunteers to glyphosate (purity not given) at a concentration of 0.36 mg/L for 1 hour. Sperm DNA fragmentation increased slightly from exposure to glyphosate, but this increase was not significant.

De Almeida et al. (2018) [180] exposed human cell lines MCF7, MDA-MB-231 (breast cancer cell lines) and HEC1A cells (endometrial cancer) for 4 hours to glyphosate (99.5% purity; 500 and 1000 µg/mL), Roundup (360 g/L glyphosate; 500 and 800 µg/ml) and Wipeout (500 g/L glyphosate; 500 and 800 µg/mL). HEC1A and MDA cells had a significant increase in DNA damage (comet assay) at both concentrations for all three compounds while MCF7 cells showed no DNA damage at either exposure. There was little difference between glyphosate, Roundup and Wipeout. Cell viability was not impacted at these exposures for these cell lines.

Santovito et al. (2018) [223] obtained human peripheral blood lymphocytes from 4 female and 2 male volunteers and exposed them to glyphosate (purity not given) for 52 (CA test) or 72 (micronucleus) hours at doses of 0.5, 0.1, 0.05, 0.025 and 0.0125 µg/mL. Glyphosate induce significant increases CAs at all concentrations except the smallest concentration (increased, but not statistically significant). The same results were seen

for micronuclei formation. Increases were seen for nucleoplasmic bridges and nuclear buds for all doses, but none were significantly different from control.

Wozniak et al. (2018) [224] obtained peripheral blood mononuclear cells from aged 18 to 55 (number not given) and exposed them to glyphosate (95% purity), Roundup 360 Plus (glyphosate 360 g/L) and AMPA (95% purity) for 24 hours at glyphosate equivalent concentrations of 0.001 to 10 µM (Roundup) and 0.5-1000 µM (glyphosate) and at concentrations of 0.5 to 1000 µM for AMPA. Significant increases in DNA damage (comet assay) were seen above a concentration of 5 µM for Roundup, above 250 µM for glyphosate and above 500 µM for AMPA. Similar findings were seen for increases in oxidized DNA pyrimidines and purines for the three compounds.

7.2.1.3 Genotoxicity in Non-Human Mammals (in vivo)

Bolognesi et al. (1997)[214] exposed groups of three Swiss CD-1 male mice by Intraperitoneal (IP) injection with a single dose of glyphosate (99.9% purity, 300 mg/kg) or Roundup (900 mg/kg, equivalent to 270 mg/kg glyphosate). Animals were sacrificed at four and 24 hours after injection and livers and kidney were removed to obtain crude nuclei from the adhering tissues. Both tissues demonstrated significant increases in DNA single-strand breaks ($p<0.05$) at four hours for both glyphosate and Roundup with no discernable difference between the responses. At 24 hours, the presence of strand breaks was reduced and no longer statistically significant from controls.

Peluso et al. (1998)[225] exposed groups of six (controls, lowest doses of glyphosate-salt and Roundup) or three Swiss CD-1 mice (males and females, specific numbers not specified, liver and kidney tissues combined for analysis) to the isopropylammonium salt of glyphosate or Roundup (30.4% isopropylammonium salt of glyphosate) for 24 hours. DNA adducts (^{32}P-DNA post labeling) were not evident in mice exposed to the glyphosate-salt alone in either liver or kidney, but were present in liver and kidney at all tested doses of Roundup showing a dose-response pattern.

Rank et al. (1993)[226] exposed male and female NMRI mice (three to five per sex) to glyphosate isopropylamine salt (purity not specified) and Roundup (480 g glyphosate isopropylamine salt per liter) by intraperitoneal injection. After 24 or 48 hours (only 24 hours for Roundup), polychromatic erythrocytes from bone marrow were extracted and micronuclei counted from a sample of 1000 cells. No significant increases were seen for any concentration in glyphosate-exposed animals (100, 150 and 200 mg/kg) or Roundup-exposed animals (133 and 200 mg/kg glyphosate equivalent dose). The positive controls, while not statistically significant, showed an increase in micronuclei.

Bolognesi et al (1997)[214] exposed groups of three, four or six male Swiss CD-1 mice to glyphosate (99.9% purity) and Roundup (30.4% glyphosate) by intraperitoneal injection in two equal doses given 24 hours apart. After six or 24 hours following the last exposure, polychromatic erythrocytes from bone marrow were extracted and micronuclei counted from a sample of 1000 cells. Mice given two doses of 150 mg/kg of glyphosate showed a non-significant increase in micronuclei at 6 hours and a significant increase at 24 hours. In contrast, mice given two doses of 225 mg/kg glyphosate equivalent of Roundup showed a significant increase in micronuclei at both six and 24

hours. The relative differences in mean absolute increase (subtract mean response in controls) in micronucleii between glyphosate and Roundup at 24 hours was 3.6 whereas the relative difference in glyphosate equivalent dose was 1.5 indicating a greater effect of the glyphosate formulation.

Manas et al. (2009)[209] exposed groups of male and female Balb C mice (group size not given, tissues combined for analysis) to glyphosate (96% purity) by intraperitoneal injection in two equal doses given 24 hours apart. Twenty-four hours post exposure, polychromatic erythrocytes from bone marrow were extracted and micronuclei counted from a sample of 1000 cells. No significant increases were seen at doses of 50 mg/kg and 100 mg/kg in glyphosate-exposed animals but a significant increase was seen at 400 mg/kg. The positive controls showed a statistically significant increase in micronuclei (roughly three times the control rate).

Dimitrov et al. (2006)[227] exposed groups of eight male C57BL mice (tissues combined for analysis) to Roundup (41% glyphosate) via gavage at a dose of 1080 mg/kg. At 6, 24, 72, 96, or 120 hours post exposure, polychromatic erythrocytes from bone marrow were extracted and micronuclei counted from a sample of 4000 cells (500 per animal). No significant increases were seen. They also looked for chromosomal damage in these animals and saw no significant increases. The positive controls showed a statistically significant increase in micronuclei.

Prasad et al. (2009)[228] exposed groups of 15 male Swiss CD-1 mice to Roundup (30.4% glyphosate) by IP injection at doses of 25 and 50 mg/kg. At 24, 48 or 72 hours post exposure, polychromatic erythrocytes from bone marrow were extracted and micronuclei counted from a sample of 2000 cells per animal, five animals per sacrifice. Micronucleii counts were significantly increased ($p<0.05$) at all doses at all times relative to controls. In addition, the number of cells with chromosomal aberrations was significantly increased for all doses at all times. The control rate of micronuclei was similar to that of **Bolognesi et al. (1997),** but about 50% greater response for a dose that was approximately 10 times smaller.

Grisolia et al. (2002)[229] exposed groups of Swiss mice (sex and sample size not given) to Roundup (480 g glyphosate isopropylamine salt per liter) by IP injection at doses of 50, 100 and 200 mg/kg Roundup in two doses separated by 24 hours. At 24 hours post exposure, polychromatic erythrocytes from bone marrow were extracted and micronuclei counted from a sample of 2000 cells per animal. Micronuclei counts were not increased at any dose. This exposure appears to be the same formulation of Roundup used in the study by **Rank et al. (1993)** which was also negative.

Coutinho do Nascimento and Grisolia (2000)[230] exposed groups of six male mice (strain not given) to Roundup (% glyphosate not given) by IP injection at doses of 50, 100 and 200 mg/kg in two doses separated by 24 hours. At 24 hours post exposure, polychromatic erythrocytes from bone marrow were extracted and micronuclei counted from a sample of 1000 cells per animal. A significant increase in micronuclei were seen at a dose of 85 mg/kg. No increase was seen at 42 or 170 mg/kg.

Cavusoglu et al. (2011)[160] exposed groups of six Swiss albino mice by IP injection with a single dose of glyphosate formulation (RoundupUltra Max, 450 g/l glyphosate, 50 mg/kg glyphosate equivalent dose). Animals were sacrificed at three days after injection. Micronuclei in normochromatic erythrocytes were counted from a sample of 1000 cells per animal. There was a significant increase in micronuclei in erythrocytes ($p<0.05$). *G. bilboa* eliminated these effects.

Chan and Mahler (1992)[231] exposed groups of 10 male and female $B6C3F_1$ mice to glyphosate (98.6% purity) in feed at doses of 0, 507, 1065, 2273, 4776, and 10780 mg/kg in males and 0, 753, 1411, 2707, 5846, and 11977 mg/kg in females for 13 weeks. At sacrifice, polychromatic erythrocytes from peripheral blood were extracted and micronuclei counted from a sample of 10,000 cells. No significant increases were seen at any of the tested doses.

Li and Long (1988)[232] exposed groups of 18 male and female Sprague-Dawley rats to glyphosate (98% purity) by IP injection at a dose of 1000 mg/kg. At 6, 12 and 24 hours post treatment, 6 animals of each sex were sacrificed and polychromatic erythrocytes from bone marrow were extracted and micronuclei counted from a sample of 50 cells per animal. The percentage of cells with chromosomal aberrations was not increased at any time point following exposure.

Milic et al. (2018) [233] exposed groups of 5 male Wistar rats to glyphosate (purity ≤100%) at doses of 0, 0.1, 0.5, 1.75 and 10 mg/kg/day by oral gavage (in phosphate-buffered saline) for 28 days. One day after the final exposure, animals were sacrificed, tissues were examined and liver and blood samples taken for analysis. Comet tail lengths and mean tail intensity for both tissues were significantly increased in all dose groups except for mean tail intensity for the two highest doses in blood. These doses are near the established regulatory guideline doses in humans.

7.2.1.4 Genotoxicity in Non-Human Mammalian Cells (in vitro)

Li and Long (1988)[232] incubated Chinese hamster ovary cells (CHO-K1BH4) with glyphosate (98% purity) for three hours at concentrations of 5, 10, 50 and 100 mg/ml. Cells were then plated using 200 cells per sample in triplicate and incubated for 8-12 days. Colonies were then counted and results expressed as mutant frequency. No positive results were seen in any experimental group with or without S9 activation. It is not clear why there is such a large difference in the incubation times in the various groups in this experiment, nor is it clear which groups incubated longer. In a second study in the same publication, non-induced primary rat hepatocytes (Fischer 344) were incubated with seven concentrations of glyphosate (12.5 ng/ml to 125 µg/ml) for 18-20 hours. No significant increases were seen for net grains per nucleus at any exposure concentration. There was a four-fold increase in the lowest exposure groups relative to controls and then every other treated group was below the control response. This is a very unusual finding and could be due to the way in which the data is adjusted for net grains in cytoplasm. The authors calculated net grains per nucleus by subtracting the highest cytoplasmic count from the nuclear count; if cytoplasmic count is increased by glyphosate this could bias the findings making any increase in nuclear count disappear.

No data is provided to resolve this issue.

Roustan et al. (2014)[234] incubated Chinese hamster ovary cells (CHO-K1) with glyphosate (purity not provided) for three hours at concentrations of 2, 5, 10, 15, 17.5, 20, and 22.5 mg/ml. Cells were then plated using 200 cells per sample in triplicate and incubated for 24 hours. For each exposure concentration, 2000 bi-nucleated cells were examined for micronuclei. No positive results were seen in any experimental group without S9 activation but the four highest exposure groups were significant with a clear concentration-response pattern when S9 activation was present.

Lioi et al. (1998)[215] exposed lymphocytes from three unrelated healthy cows to glyphosate (>98% purity) for 72 hours to concentrations of 3, 14.4 and 28.7 µg/ml without S9 activation. Chromosomal aberrations scored from 150 cells were significantly increased ($P<0.05$) for all exposure concentrations of glyphosate with a clear concentration-response pattern. Similarly, SCEs per cell were increased at all concentrations ($p<0.05$) but no concentration response pattern was evident.

Sivikova and Dianovsky (2006)[235] exposed lymphocytes from two healthy young bovine bulls to glyphosate formulation (62% glyphosate) for 2, 24 and 48 hours using concentrations of 4.7, 9.5, 23.6, 47.3, 94.6 and 190 µg/ml without S9 activation. Chromosomal aberrations scored from 100 cells were not significantly increased ($P<0.05$) without S9 activation for any 24-hour exposure concentration of glyphosate (2- and 48-hours exposures were not done). SCEs per cell were increased at all 24-hour exposure concentrations ($p<0.05$) except the lowest concentration. At 48-hours, significant increases of SCEs per cell were seen at concentrations at or above 47.3 µg/ml (2-hour exposures were not done). Finally, after two hours of exposure with S9 activation, significant effects were seen at 5 and 10 µg/ml but not at 15 µg/ml (24- and 48-hour exposures were not done for S9 activation).

Holeckova (2006)[236] exposed lymphocytes from two healthy young bovine bulls to glyphosate formulation (62% glyphosate) for 24 hours to concentrations ranging from 28 to 1120 µmol/L without S9 activation. A significant increase in polyploidy was observed at 56 µmol/L, all other comparisons were without significance. However, this one finding cannot be easily dismissed because all exposure groups above this concentration had too few cells for evaluation. This study did not consider S9 activation.

7.2.1.5 Genotoxicity in Non-Human Systems (in vivo and in vitro)

Four studies[208, 237-239] in fish have seen positive results for genotoxicity (DNA strand breaks, different assays) following exposure to glyphosate. In addition, one study[240] in oyster sperm and embryos exposed to glyphosate saw no increase in DNA damage (comet assay) and one study[241] in two strains of Drosophila melanogaster showed an increase in mutations (wing spot test) at the higher doses of exposure.

Seventeen studies[229, 237, 239, 242-255] in multiple fish species evaluated the relationship between various glyphosate formulations and genotoxicity with all studies showing positive results for various endpoints (DNA strand breaks, micronucleus

formation, and chromosomal aberrations). Two of the studies[242, 244] were negative for micronucleus formation after exposure to glyphosate formulations and one of these[242] was also negative for chromosomal aberrations but both were positive in other markers of genotoxicity. Two studies[256, 257] demonstrated genotoxicity (DNA strand breaks, micronuclei) in caiman from *in-vivo* exposure to a glyphosate formulation and one study [258] showed an increase in chromosomal aberrations (but not MN or NA) in caiman exposed in the egg to a glyphosate formulation. Three studies[259-261] demonstrated genotoxicity (DNA strand breaks, micronucleus formation) in frogs or tadpoles from exposure to glyphosate formulations. One study[240] in oyster sperm and embryos, one study[262] in clams and one study[263] in mussels exposed to a glyphosate formulation saw no increase in DNA damage (comet assay). One study[264] in snails saw increased DNA damage (comet assay) following exposure to a glyphosate formulation. Two studies[265, 266] in worms saw mixed results for DNA damage (comet assay) with one of these studies[265] showing a positive result for micronucleus formation. One study[267] in Drosophila melanogaster showed an increase in sex-linked recessive lethal mutations. One study in crabs [268] showed an increase in DNA damage (comet assay). One study in peripheral blood lymphocytes of big hairy armadillos [269] showed increases in CAs and SCEs to three different doses of a glyphosate formulation in vivo and a second study [270] with four dose groups showed significant increases in-vivo. Freshwater shrimp exposed to a glyphosate formulation showed increased DNA damage (comet assay, micronucleus assay) [271].

In the published literature, five studies evaluated the impact of glyphosate in *in vitro* systems. Two of these studies[272, 273] looked at genotoxicity of glyphosate in combination with UVB radiation and saw significant increases in DNA strand breaks (FADU assay) in bacteria without metabolic activation. One study[274] in eukaryote fish saw a significant increase in DNA strand breaks (comet assay) without S9 activation. Another study[232] showed no increase in reverse mutations in two strains of bacteria with and without S9 activation.

Williams et al. (2000)[275] summarized the literature regarding the use of reverse mutation assays in *S. typhimurium* (Ames Test). Four studies using glyphosate and five studies of glyphosate formulations were all negative. They cited one study[226] of a glyphosate formulation that was positive with S9 activation and negative without S9 activation. However, this study was positive with S9 activation in TA100 cells, negative with S9 activation in TA98 cells, negative without S9 activation for TA100 cells and positive without activation for TA98 cells. They also summarized two studies of glyphosate in *e. coli* that were negative with and without activation.

Two additional studies[232, 276] of glyphosate using reverse mutation assays are available from the scientific literature, both of which are negative.

7.2.1.6 Regulatory Studies

EFSA[55] cited 14 reverse mutation assays in *S. typhimurium* (Ames Test), most of which were tested in strains TA 98, 100, 1535, 1537 (Table B.6.4-1). All 14 studies are listed as

negative by EFSA. Actual data is provided for only one of the 14 studies and this study is clearly negative. **EPA**[54] cited 27 reverse mutation assays in *S. typhimurium* (Ames Test), most of which were tested in strains TA 98, 100, 1535, 1537 (EPA Table 5.1). All 27 studies are listed as negative. No data is provided for any of the studies. **Kier and Kirkland (2013)[61]** cited results from 18 bacterial reverse mutation assays of glyphosate and 16 of glyphosate formulations. Tabulated results and background information were provided for all 34 studies. Six studies of glyphosate alone demonstrated positive findings in one or more groups.

EFSA[55] cites three studies of gene mutations in mammalian cells, all of which are listed as negative (EFSA Table B.6.4-5), two use the mouse lymphoma assay, and one uses the Chinese hamster ovary cell/hypoxanthine-guanine phosphoribosyl transferase (CHO/HGPRT) mutation assay. **EPA**[54] cites four studies, three of which appear to be the same as those cited by EFSA (EPA Table 5.2) and the fourth is another mouse lymphoma assay. All four are listed as negative. **Kier and Kirkland (2013)**[61] cite two of the mouse lymphoma studies and provide tabulated data. Neither study shows any indication of a statistically significant increase in mutation frequency at the thymidine kinase locus of L5178 mouse lymphoma tk(+/-) cells.

EFSA[55] cites one *in vitro* study of DNA damage and repair in mammalian cells which is listed as negative (EFSA Table B.6.4-6). This study is of unscheduled DNA synthesis (UDS assay) in primary rat lymphocytes. They also list five studies of chromosome aberrations (EFSA Table B.6.4-8), which are characterized as negative. Two studies are in human lymphocytes and two are in Chinese hamster lung (CHL) cells. Data for one of the studies in CHL is provided in tabular form and is clearly negative. **EPA**[54] cites eight *in vitro* studies of chromosome aberrations in mammalian cells (EPA Table 5.3); two of these studies match studies in the EFSA report. Four of the studies are from the literature[209, 215, 235, 277] and are reviewed above. Surprisingly, EPA refers to the study by **Manas et al. (2009)**[209] as negative although it was clearly positive in the comet assay., Additionally, EPA refers to the study by **Sivikova and Dainovsky (2006)**[235] as negative even though they saw clear effects of glyphosate on SCEs. Basically, all four of the literature studies cited by EPA are positive yet EPA lists only two of the four as positive. The remaining four studies are noted as negative; however, no data is supplied for these studies. **Kier and Kirkland (2013)**[61] cites eight literature studies (all reviewed above) and three regulatory studies with glyphosate exposure. The three regulatory studies are listed as negative, and the data are available as a table in the supplement material to **Kier and Kirkland (2013)**; these studies are negative at all tested concentrations in CHL cells; one matches the study data provided by EFSA[55].

EFSA[55] cites nine micronucleus assays, three in Swiss Albino mice, two in NMRI mice, two in CD-1 mice, one in Sprague-Dawley rats, and one in CD rats (EFSA Table B.6.4-12). They list one study in Swiss Albino mice as weakly positive in males, one study in CD-1 mice as positive at the highest dose (data for this study is provided) and all other studies as negative. They discard one study with low doses in male Swiss mice, but the tables provided for this study show a clearly significant result at the highest dose used (30 mg/kg) and clear dose-response. They provide data for two of the negative studies

which indicate these studies were indeed negative. **EPA**[54] (EPA Table 5.5) cites 20 micronucleus assays, four are available in the scientific literature and three are reviewed above (the fourth reference[278] was unavailable to me at the time of preparation of this report). The remaining 16 studies include six studies in Swiss Albino mice, four studies in CD-1 mice, three studies in NMRI mice, two studies in Sprague-Dawley rats and one study in Wistar rats. Since EFSA does not provide names associated with their micronucleus studies, I cannot determine if any of the studies cited by the EPA are the same as those cited by EFSA. EPA lists two of the literature studies as positive and two as negative (matching my reviews for the three studies I have access to) and all but one of the regulatory studies as negative (the one positive study was in Swiss-Albino mice). **Kier and Kirkland (2013)**[61] cite 12 regulatory micronucleus assays of glyphosate and provide data tables for all 12. All 12 of these studies are cited by EPA. **Kier and Kirkland (2013)** list 11 studies as negative and one as inconclusive. However, four of the studies show positive effects in at least one sex-by-treatment group. One of these four studies they list as inconclusive and the remaining three studies are determined to be negative because the response is within the range of the historical controls. As was discussed for the animal carcinogenicity studies, the correct group to use is the concurrent control. **Kier and Kirkland (2013)**[61] also cite 12 regulatory studies and three literature studies where animals are exposed to a glyphosate formulation. Two of the literature studies are reviewed above and the remaining study[278] was unavailable. Data for the 12 regulatory studies are all provided in tables by **Kier and Kirkland (2013)** and show two positive studies in CD-1 mice and negative studies for the remaining 10.

7.2.1.7 Summary for Genotoxicity

This is a complicated area from which to draw a conclusion due to the diversity of the studies available (there are multiple species, multiple strains within a species, multiple cell types from multiple species, differing lengths of exposure, differing times of evaluation after exposure, differing exposures, numerous markers of genotoxicity, and finally both glyphosate and multiple different glyphosate formulations). There are three studies that evaluate the genotoxicity of glyphosate in humans directly, 36 experiments in eight strains of mice, four studies in rats, 14 studies in human lymphocytes and 18 studies in other human cells, 12 studies in non-human mammalian cell lines (two using mouse cells, five using hamster cells, two using rat cells and three using cells from cows), a large number of studies in a wide variety of non-mammalian species, and a plethora of studies, mostly identical, in bacteria.

Some conclusions are straightforward"; glyphosate does not appear to cause reverse mutations for histidine synthesis in *Salmonella typhimurium*, regardless of whether these reverse mutations are due to frameshift mutations or point mutations. I am cautious in this determination because there were several studies with positive results, but no clear pattern is evident. There is ample evidence supporting the conclusion that glyphosate formulations and glyphosate can cause genotoxicity in non-mammalian animal species. This clearly indicates that both glyphosate and the formulations are able to cause injury to DNA. So while findings of genotoxicity in these species do not speak directly to the hazard potential in humans, they do support a cause for concern.

The more important studies are those that have been done using mammalian systems, human cells and direct human contact. Table 21 summarizes these studies in a simple framework that allows all of the experimental data to be seen in one glance. This table does not address the subtlety needed to interpret any one study, but simply demonstrates when a study produced positive versus negative results.

Clearly, for *in vitro* evaluations in human cells, the majority of the studies have produced positive results. There was only one regulatory study evaluating glyphosate genotoxicity in human lymphocytes from healthy volunteers and that study was negative. The study was not significantly different from the other nine studies in this category, eight of which produced positive results. The majority of these studies used either the comet assay (a simple way for measuring any type of DNA strand break) or methods that counted specific types of strand breaks in the cells (e.g. SCEs, micronuclei, nuclear buds and nucleoplasmic bridges). From these assays, we can conclude there is DNA damage. For glyphosate formulations, there are only three studies in humans *in vivo*, two of which were positive. In human cell lines, there are 5 positive studies and no negative studies.

The magnitude of the concentrations used in these studies could potentially lead to false positives if the glyphosate is causing cytotoxicity in the cells. All studies using the comet assay were positive with no study showing a negative response above 10 µg/ml and mixed results below that with positive results at 0.12 and 3.5 µg/ml and negative results at 2.91 and 10 µg/ml. In general, the comet assays provide strong support for genotoxicity.

The studies that directly addressed specific types of strand breaks in cells following exposure to glyphosate showed markedly different responses across the various concentrations used. **Manas et al. (2009)** saw no changes in chromatid breaks, chromosome breaks, chromatid gaps, chromosome gaps, dicentrics, acentric fragments or endoreduplication over the range of concentrations 3.4-1015 µg/ml. In contrast, **Lioi et al. (1998)** saw changes in SCEs over concentrations ranging from 1.4 to 8.7 µg/ml and **Kim et al. (2016)** saw increases in SCEs at 0.3 µg/mL . All three studies were done in lymphocytes from volunteers. **Mladinic et al. (2009)** saw significant changes in micronuclei above 92.8 µg/ml and **Bolognesi et al. (1997)** saw positive changes in SCEs above 1000 µg/ml but not at 330 µg/ml. **Kasuba et al. (2017)** saw increases in nuclear buds and micronuclei above 0.5 µg/mL and **Santorito et al. (2018)** saw changes in CAs and SCEs above 0.1 µg/mL. While changes have been seen in six of the seven studies, the actual concentrations in which the changes are seen is not consistent across studies. I conclude that glyphosate causes DNA strand breaks, which is indicative of genotoxicity.

The micronucleus assays in rodents examining glyphosate genotoxicity are either all positive in one strain or all negative in one strain with the exception of the three studies in CD-1 mice and four studies in Swiss Albino mice. For the positive studies, we can ask the question of whether, in this strain, the actual number of micronuclei are consistent.

Table 21: Summary of in vivo and in vitro genotoxicity studies of glyphosate and glyphosate formulations in mammals[1]

In vivo or *in vitro*	Species	Cell type or tissue	Glyphosate[2]		Glyphosate Formulations	
			Number Positive	Number Negative	Number Positive	Number Negative
In vivo	Humans	Peripheral blood			2	1
in vitro	Humans	lymphocytes	8	2(1)	4	
		semen		1		
		cell lines	8	1	6	2
In vivo	Swiss CD-1 Mouse	Liver/Kidney	1	1	2	
	Wistar Rats	Liver/blood	1			
In vivo (micro-nucleus assay)	NMRI mouse	Erythrocytes		4(3)		2(1)
	Swiss CD-1 mouse		1		2	
	Balb C mouse		1			
	$B6C3F_1$ mouse			1		
	Swiss mouse		1(1)			3(2)
	CD-1 mouse		2(2)	1(1)	2 (2)	6 (6)
	Swiss albino mouse		1(1)	3(3)	1	
	C57BL mouse					1
	Mouse (not specified)				1	
	Rats (all)			2(1)		1(1)
In vitro	Mouse	L5178 lymphoma		2(2)		
	Chinese hamster	Lung		3(3)		
	Chinese hamster	ovary	1	1		
	Fischer rat	liver		1		
	Rat	Lymphocytes		1(1)		
	Bovine	Lymphocytes	1		2	

[1]each entry in the table corresponds to a single study where a study is positive if at least one valid positive finding emerged from the study $p<0.05$; entries in the table are only for studies where data was available to review including data from EFSA[55] and Kier and Kirkland (2000)[61]; [2]numbers are the total number of studies in this category, numbers in parentheses are the subset of studies that are regulatory studies

In Swiss Albino mice, all four studies using glyphosate were done with males and

females. Exposures were by oral gavage for the positive study (in female mice) and IP injection by the negative studies. The positive study was at 5000 mg/kg and the highest dose in any of the negative studies was 3024 mg/kg. Finally, the control response in the positive study was 6.7 micronucleated PCE per 1000 PCE whereas the controls in the three negative studies were between 0 and 0.6 micronucleated PCE per 1000 PCE. Any of these differences could easily explain the differences in response so the positive result in Swiss Albino mice should be accepted.

For CD-1 mice, the one negative micronucleus study was by oral gavage in males and females at a single dose of 5000 mg/kg. One of the positive studies was also by oral gavage in males at a single dose of 2000 mg/kg. Because of the nature of statistical noise, these two studies could both occur whether there is a true effect or not. For the other positive study, the dose was by IP injection in male mice with a positive response at 600 mg/kg that was more than double the response of the controls. These data support the finding that glyphosate can cause micronuclei in male CD-1 mice, which is indicative of genotoxicity.

The remaining *in vitro* assays in mammalian cells exposed to glyphosate show mixed results. The mouse lymphoma assay and the Chinese hamster ovary assays are looking for specific mutations that will allow these cells to grow in culture. The Chinese hamster lung, the two rat assays and the assay in bovine lymphocytes are measuring DNA damage and provide mixed results. In general, these responses appear to be negative with the exception of those seen in bovine lymphocytes that appear to show a positive increase in SCEs following exposure to glyphosate.

For glyphosate formulations, the main difference between the findings for glyphosate and those for the glyphosate formulations is the direct evidence for genotoxicity in humans and the micronucleus assays in Swiss mice. The observation of genotoxicity in humans following exposure to glyphosate formulations must carry the greatest weight in the overall analysis and two of the three studies were positive with the strongest study by **Bolognesi et al. (2009)**[62] showing the strongest response.

For the Swiss mouse studies of micronuclei, the fact that all three studies are negative for glyphosate formulations while one study is positive for glyphosate creates a clear disagreement. The positive study is an oral gavage study with an effect seen in male mice at 30 mg/kg/day. The two negative regulatory studies for glyphosate formulations were done at 2000 mg/kg (about 500 mg/kg glyphosate equivalent), were also oral gavage studies and were replicates done in the same laboratory at different times. The remaining negative study used glyphosate formulation doses of 50-200 mg/kg (25-100 mg/kg glyphosate equivalent) but was done by intraperitoneal injection. With the exception of the different routes of exposure, the differences between these studies cannot be resolved.

In this case, a pooled analysis of the data is not possible because in almost every case, no one study is a clear replicate of another. Instead, the appropriate approach would be to do a meta-analysis and evaluate which aspects of the experimental designs are important to producing positive findings of genotoxicity. The studies with the most data

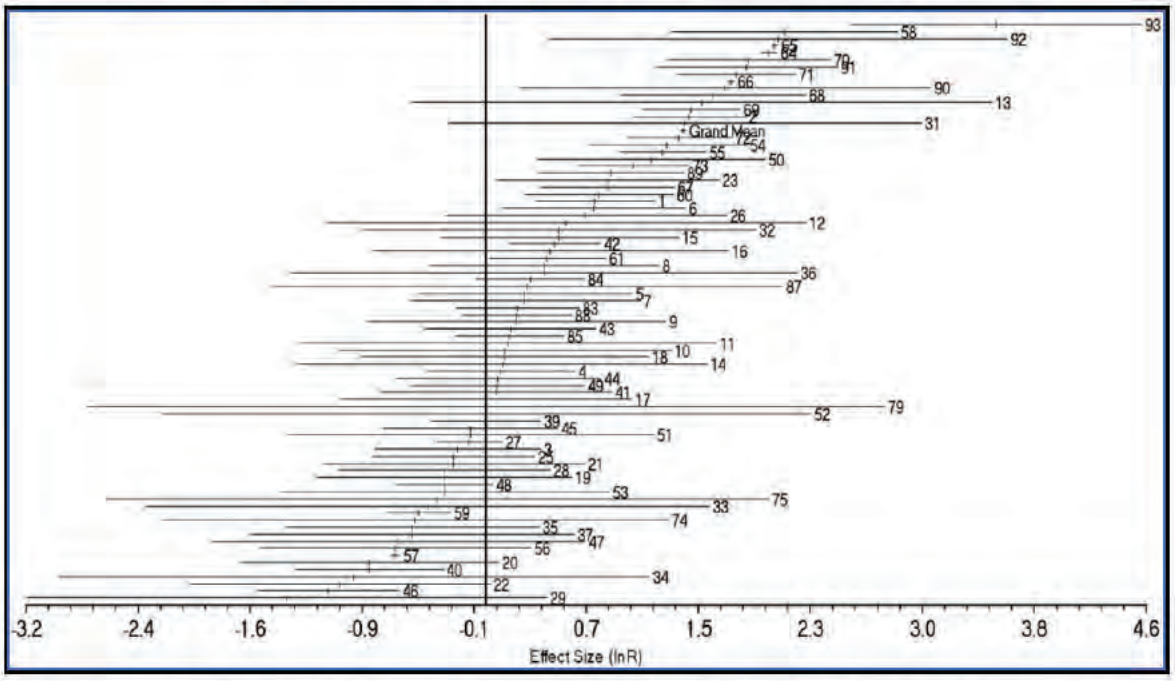
for this type of analysis are the various *in vivo* assays of micronucleus formation. **Ghisi et al. (2016)**[63] did a systematic search to identify all published studies evaluating the ability of glyphosate or glyphosate formulations to induce micronuclei *in vivo*. The authors also used the data from **Kier and Kirkland (2013)**[61] summarized above. An experiment, in their evaluation, was defined by sex/species/route/form of glyphosate so that some studies doing both sexes using glyphosate and a glyphosate formulation will enter multiple times into the analysis. They identified 93 experiments from which it was possible to do a meta-analysis. Data were extracted for each study and the log ratio of the mean of each experimental group to the mean control response (E+) was used to evaluate effect sizes in the meta-analysis. For this meta-analytic mean, a value below zero suggests no genotoxicity while a value above zero suggests increased genotoxicity. A test of heterogeneity (Cochran's Q statistic discussed earlier for the epidemiological data) was also evaluated.

Figure 3 is a reprint of Figure 1 from the study by **Ghisi et al. (2016)**[63] and is a forest plot from all studies they evaluated for glyphosate and glyphosate formulations. It is clear from this plot that the predominant response is positive in these data with an overall grand mean response across all studies of E+=1.37 and a 95% confidence interval of (1.356-1.381) (this is highly statistically significant with a $p<0.0001$). The Qt value for the grand mean was also statistically significant suggesting there are other explanatory variables in the data that would help to explain the overall variance.

Categorical variables were then used to make comparisons across the various strata in the data to identify which experimental conditions show the largest impacts on the mean response. Mammalian species presented a higher mean effect (E+=1.379; 1.366-1.391) than non-mammalian species (E+=0.740; 0.641-0.840). Glyphosate formulations showed a greater mean response (E+=1.388; 1.375-1.400) than did glyphosate (E+=0.121; 0.021-0.221), but both were significantly greater than zero. The mean response in studies using only male animals (E+=1.833; 1.819-1.847) was significantly different from zero as were studies using both males and females (E+=0.674;0.523-0.825) whereas the mean response in studies using only females (E+0.088; -0.153-0.328) was not. Peer-reviewed studies had higher mean response (E+=1.394; 1.381-1.407) compared to regulatory studies (E+=0.114; 0.027-0.202), but both means were significantly greater than zero, indicating an overall genotoxic effect. Other variables were examined such as length of exposure and magnitude of exposure that had very little impact on the overall findings.

The meta-analysis by **Ghisi et al. (2016)**[63] provides strong support for the hypothesis that exposure to glyphosate and glyphosate formulations increases the formation of micronuclei *in vivo*. This means that glyphosate and glyphosate formulations are damaging DNA in living, functioning organisms with intact DNA repair capacity strengthening the finding that glyphosate is genotoxic to humans.

Figure 3: Forest plot of studies evaluating micronucleus frequency in glyphosate exposure, arranged by effects size [Reprinted from Ghisi et al. (2016)[63]].



The plot shows the estimate of the response ratio and 95% confidence interval (CI) of each experiment included in the meta-analysis. The number beside the bars represents the reference number of each experiment as in Table 1 of Ghisi et al. (2016)[63]. Grand Mean is the overall mean effects size of all studies. [Reprinted from Ghisi et al. (2016)[63]]

From a simply statistical perspective, there is another way in which one can decide if the positive findings in the micronucleus assays in the mice are due to chance. For the glyphosate studies, if one adds up all of the individual experimental groups, there are 79 total groups which correspond to 79 statistical tests. Assuming the critical testing level is 0.05 for all of the tests, one would expect to see just under four positive findings, yet six are observed. For the glyphosate formulations, there were 70 experimental groups so one expects 3.5 positive findings yet 12 are observed ($p<0.01$). Overall, there were a total of 149 experimental groups examined in mice for micronucleus formation and we observed 18 (7.5 expected, $p<0.01$). Repeating this analysis on the basis of studies instead of experimental groups, there were 15 studies for glyphosate (expected number is 0.75 positive) yet six positive were observed ($p<0.01$). For the glyphosate formulations, there were 18 studies (expected number is 0.9 positive) yet six positive are observed ($p<0.01$). Now expanding to all 69 studies presented in Table 21, there were 33 positive studies, but the expectation is a mere 3.5 ($p<0.01$).

It is clear that both glyphosate and glyphosate formulations have genotoxic potential. But which is worse? Of the 86 experiments in Table 21, there were 19 experiments from 7 research publications that addressed both glyphosate and a glyphosate formulation in the same laboratory. Of these, five were negative for both glyphosate and the formulation and do not contribute to a discussion of relative potency. The remaining 14

can provide some guidance on the relative potency of glyphosate to glyphosate formulations. In **Koller et al. (2007)**[212], tail intensity for the comet assay were virtually identical when the amount of glyphosate in the formulation was compared to the results using glyphosate alone. In the same paper, micronuclei and related biomarkers were consistently higher in the glyphosate formulation by 10-20%. In **Bolognesi et al. (1997),** DNA strand breaks in liver and kidney in Swiss CD-1 mice were virtually identical under equivalent doses of glyphosate and glyphosate formulations. In their micronucleus assay, the glyphosate formulation was approximately 50% more potent. Finally, **Bolognesi et al. (1997),** in their analysis of SCEs in human lymphocytes, the glyphosate formulation was approximately twice as effective as glyphosate alone. In **Peluso et al. (1988)**[225], DNA adducts in livers and kidneys were only seen in mice treated with the glyphosate formulation, so these findings are not likely to be due to glyphosate. **Wozniak (2018)** saw a clear difference with glyphosate formulations showing greater impacts on DNA damage than pure glyphosate. **De Almeida (2018)** showed very little difference between glyphosate and glyphosate formulations on genotoxicity. The data suggest a small increase in the potential for genotoxicity for glyphosate formulations relative to the genotoxicity one would see with glyphosate alone.

In summary, the data support a conclusion that both glyphosate and glyphosate formulations are genotoxic. Thus, there is a reasonable mechanism supporting the increases in tumors caused by glyphosate and glyphosate formulations in humans and animals.

7.2.2 Oxidative Stress

Oxidative stress refers to an imbalance between the production of reactive oxygen species (free radicals) in a cell and the antioxidant defenses the cell has in place to prevent this. Oxidative stress has been linked to both the causes and consequences of several diseases[64-69] including cancer[70-75]. Multiple biomarkers exist for oxidative stress; the most common being the increased antioxidant enzyme activity, depletion of glutathione or increases in lipid peroxidation. In addition, many studies evaluating oxidative stress used antioxidants following exposure to glyphosate to demonstrate that the effect of the oxidative stress can be diminished.

7.2.2.1 Oxidative Stress in Human Cells (in vitro)

Mladinic et al. (2009)[207] examined the induction of oxidative stress from exposure to glyphosate (98% purity) in lymphocytes from three healthy human donors (questionnaires were used to exclude other genotoxic exposures) at concentrations of 0.5, 2.91, 3.5, 92.8 and 580 µg/ml. Cells with and without S9 activation saw increases in total antioxidant capacity at only the highest dose for cells without S9 activation although a clear concentration response pattern was seen with S9 activation.

Kwiatkowska et al. (2014)[279] examined the induction of oxidative stress from exposure to glyphosate (purity not given) in erythrocytes obtained from healthy donors in the Blood Bank of Lodz, Poland. Erythrocytes were exposed to concentrations of 1.7, 8.4, 17, 42.3, 85 and 845 µg/ml and incubated for 1 hour. Oxidative stress (oxidation of

dihydrorhodamine 123) was significantly increased at 42.3, 85 and 845 µg/l with a clear concentration-response pattern.

Chaufan et al. (2014)[280] examined the induction of oxidative stress from exposure to glyphosate (95% purity) and Roundup UltraMax (74.7% glyphosate) in HepG2 cells (human hepatoma cell line). Exposure concentrations were 900 µg/ml for glyphosate and 40 µg/ml for the glyphosate formulation. After incubation for 24 hours, oxidative stress (expressed as the activity of superoxide dismutase (SOD), catalase (CAT), glutathione (GSH) and glutathione-S-transferase (GST)) was significantly increased ($p<0.0\text{-}5$) for the glyphosate formulation (increased SOD activity) but not for glyphosate alone.

Coalova et al. (2014)[281] examined the induction of oxidative stress from exposure to a glyphosate formulation (Atanor, 48% glyphosate) or with a surfactant (Impacto) in Hep-2 cells (human epithelial cell line). Exposure concentrations were 376.4 µg/ml for Atanor, 12.1 µg/ml for Impacto and 180.2 µg/ml for a mixture of the two. After incubation for 24 hours, oxidative stress (measured as activity of SOD, CAT, GSH, and GST) was significantly increased for Impacto, Atanor and the mixture (CAT and GSH only, $p<0.05$ or $p<0.01$).

Gehin et al. (2005)[282] examined the induction of oxidative stress from exposure to glyphosate (purity unknown) and a glyphosate formulation (Roundup 3 plus, 21% glyphosate) in HaCaT cells (human keratinocyte cell line). Glyphosate induced cytotoxicity in the cells which was reduced or eliminated by antioxidants. The authors attributed the cytotoxicity to oxidative stress.

Elie-Caille et al. (2010)[165] examined the induction of oxidative stress from exposure to glyphosate (purity unknown) in HaCaT cells (human keratinocyte cell line). Exposure concentrations ranged from 1700 µg/l to almost 12,000 µg/ml. Glyphosate induced cytotoxicity in the cells and increased hydrogen peroxide H_2O_2 (dichlorodihydrofluorescein diacetate assay). This study used exceptionally high concentrations that may be inducing cytotoxicity by means that are independent of the oxidative stress observed. Measuring oxidative stress using the dichlorodihydrofluorescein diacetate assay has limitations[283, 284].

George and Shukla (2013)[285] examined the induction of oxidative stress from exposure to a glyphosate formulation (Roundup Original, 41% glyphosate) in HaCaT cells (human keratinocyte cell line). Exposure concentration ranged from 1.7 µg/ml to 17,000 µg/ml and exposure was for 24 hours. Glyphosate significantly induced the formation of reactive oxygen species (dichlorodihydrofluorescein diacetate assay) at all exposures in a concentration-dependent fashion. Prior treatment of the cells with N-Acetylcysteine reduced the impact of glyphosate, but did not eliminate it. Measuring oxidative stress using dichlorodihydrofluorescein diacetate has limitations[283, 284] that affect the clear interpretation of these results.

Kasuba et al. (2017) [218] exposed human HepG2 cells in-vivo to glyphosate (analytical grade) glyphosate for 4 hours and 24 hours at doses of 0.5 µg/mL, 2.91 µg/mL and 3.5 µg/mL. There were no differences in cellular proliferation between the exposed cells

and the controls. After 4 hours of exposure, there was a significant decrease in TBARS at 2 of the three concentrations. After both 4 and 24 hours of exposure there were non-significant concentration-dependent reductions in total antioxidant activity at all concentrations. There was no change in ROS or glutathione at any concentration regardless of time.

7.2.2.2 Oxidative Stress in Non-Human Mammals (in vivo)

Bolognesi et al. (1997)[214] exposed groups of three Swiss CD-1 male mice by IP injection with a single dose of glyphosate (99.9% purity, 300 mg/kg) or Roundup (900 mg/kg, equivalent to 270 mg/kg glyphosate). Animals were sacrificed at eight and 24 hours after injection and livers and kidney were removed to obtain crude nuclei from the adhering tissues. Samples of liver and kidneys from these mice were evaluated for levels of 8-hydroxy-2'-deoxyguanosine (8-OHdG) which is a biomarker of oxidative stress[286]. There was a significant increase in the liver of 8-OHdG at 24 hours following glyphosate exposure, but not at eight hours and not in the kidney. At both eight hours and 24 hours, Roundup increased 8-OHdG in the kidneys, but the mild increase seen in the liver at 24 hours was not significant.

Cavusoglu et al. (2011)[160] exposed groups of six Swiss albino mice by IP injection of a glyphosate formulation (RoundupUltra Max, 450 g/l glyphosate, 50 mg/kg formulation). At the end of dosing, animals were fasted overnight then sacrificed. There was a significant increase in malondialdehyde in both liver and kidney and a significant decrease in GSH in liver and kidney from exposure to the glyphosate formulation. *G. bilboa* eliminated these effects.

Jasper et al. (2012)[287] exposed groups of 10 male and 10 female Swiss albino mice via oral gavage for 15 days to a glyphosate formulation (Roundup Original, 41% glyphosate, 50 mg/kg glyphosate equivalent dose). Animals were sacrificed at three days after injection. There was a significant increase in thiobarbituric acid-reactive substances (TBARS) in the liver for both male and female mice at both doses ($p<0.05$). The concentration of non-protein thiols was elevated in both dose groups for males and for the high dose only in females (no dose-response was seen for this endpoint).

Astiz et al. (2009)[288] exposed groups of four male Wistar rats by IP injection to a single dose of glyphosate (purity unknown, 10 mg/kg). Animals were injected three times per week for five weeks and then sacrificed. Thiobarbituric acid-reactive substances (TBARS assay), protein carbonyls (PCOSs), total glutathione levels, individual glutathione levels, SOD and CAT were all measured as biomarkers for oxidative stress in plasma, brain, liver and kidney. Glyphosate significantly increased TBARS in all tissues ($p<0.01$), total glutathione in brain ($p<0.01$), SOD in liver and brain ($p<0.01$) and CAT in brain. In a follow-up report[176], they demonstrate that lipoic acid eliminates or severely reduces the impacts of glyphosate on the brain.

Cattani et al. (2014)[289] exposed groups of four pregnant Wistar rats to glyphosate formulation (Roundup Original, 360 g/L glyphosate) in drinking water from gestational days 5-15 at a dose of 71.4mg/kg. Fifteen day-old pups (2 per dam) were examined for oxidative stress markers in the hippocampus. Pups had a significant increase in TBARS

($p<0.05$) and a significant decrease in GSH ($p<0.01$).

George et al. (2010)[45] exposed groups of four Swiss albino mice to a glyphosate formulation (Roundup Original, 36g/L glyphosate) at a dose of 50 mg/kg (glyphosate equivalent dose) via a single topical application. Proteomic analysis of skin from the treated animals saw alterations in SOD1, CA III and PRX II, proteins known to play a role in the management of oxidative stress.

Tang et al. (2017) [157] exposed groups of 8 male Sprague-Dawley rats to glyphosate (purity not given) at doses of 0, 5, 50 and 500 mg/kg/day by gavage for 35 days. Oxidative status in liver, kidney and blood were determined by measuring the activity of gltaumic-oxalacetic transaminase (GOT), glutamic-pyruvic transaminase (GPT), total superoxide dismutase (T-SOD), malondialdehyde (MDA), hydrogen peroxide (H_2O_2), catalase (CAT), glutathione (GSH) and glutathione peroxidase (GSH-Px). Glyphosate resulted in elevated GOT (high dose) and GPT in serum (high dose), decreased T-SOD (high dose all three tissues, mid-dose kidney) and increased MDA (significant only in mid-dose serum) in serum, liver and kidney, increase H_2O_2 (significant in high-dose liver only) and CAT (significant in high-dose serum only) in serum and liver and decrease GSH (mid-dose) and GSH-Px (mid-dose and high dose) in the kidney, all indicating an increase in oxidative stress.

Avdatek et al. (2018) [290] exposed groups of 7 male Wistar Albino rats for eight weeks to doses of glyphosate (purity not given) and resveratrol as follows; control, 20 mg/kg/day resveratrol, 375 mg/kg/day glyphosate and the combination. They measured oxidative stress in the testis using MDA, GSH, SOD and CAT activity. The group given glyphosate alone saw a significant increase in MDA and decreases in GSH (non-significant) and SOD ($p<0.05$). These were reversed by adding resveratrol.

Gallegos et al. (2018) [291] exposed groups of 10 pregnant Wistar rats to Glifloglex (48 g glyphosate per 100 cm^3) from gestational day 0 (conception) to postnatal day 21 (21 days after birth, weanling) to doses of 0, 0.65g/l and 1.3 g/l of glyphosate-equivalent dose in drinking water. Oxidative stress was assessed on postnatal day 90 using homogenized brain tissue in 5 male and 5 female pups per group by measuring MDA, CAT and GSH-Px. MDA and CAT were significantly reduced in brain tissue for both dose groups and GSH-Px was significantly increased for the highest exposure group.

7.2.2.3 Oxidative Stress in Non-Human Mammals (in vitro)

Bhardwaj et al. (2019) [292] exposed cultured healthy antral follicles from goats (obtained from a slaughterhouse) (in vitro) an unspecified glyphosate formulation (41% glyphosate) for 24, 48 and 72 hours at concentrations of 0.1, 2.0 and 4.0 mg/L glyphosate equivalents. There were significant reductions in superoxide dismutase activity, CAT activity, and GSH activity and increases in TBAR and MDA at all doses at all times. Vitamin E and vitamin D attenuated these effects.

7.2.2.4 Oxidative Stress in Non-Mammalian Systems

As for genotoxicity, oxidative stress from exposure to glyphosate and glyphosate formulations have been studied in various aquatic organisms; reviewed in **Slaninova et**

al. (2009)[293]. Many of the studies reviewed by **Slaninova et al. (2009)** showed associations with glyphosate and oxidative stress in various organs. Since that review, additional studies have been completed that also demonstrate a positive association between glyphosate and oxidative stress[239, 248-251, 271, 294-317].

7.2.2.5 Summary for Oxidative Stress

Eight studies addressed oxidative stress in human cells and another ten studies addressed it in mammalian systems. In lymphocytes and erythrocytes from healthy donors, oxidative stress was detected as low as 580 µg/ml in lymphocytes and at 42.3 µg/ml in erythrocytes. In HepG2 cells, no increased oxidative stress was seen for a single concentration of 900 µg/mL, however, in a second study, some markers of oxidative stress were significantly changed at doses as low as 2.91 µg/mL. In two studies in HaCat cells, glyphosate induced oxidative stress in a continuous model fit to the results in one study and at the lowest concentration (1700 µg/ml) in the other. The most convincing studies in human cells for oxidative stress are the two studies in human blood.

In Swiss CD-1 male mice, increased oxidative stress was seen in the liver at 24 hours, but not at four hours after injection of 300 mg/kg glyphosate. No increase was seen in the kidney. In Wistar rats, repeated IP dosing with glyphosate lead to increased oxidative stress in multiple organs using multiple biomarkers. Thus, all of the laboratory studies demonstrated oxidative stress with a significant finding in the rat study.

In Hep-G2 cells, a glyphosate formulation demonstrated a robust increase in oxidative stress at 40 µg/ml. Given the negative response in this cell line for glyphosate alone, it must be concluded that this response is not due to glyphosate. In HEP-2 cells, a glyphosate formulation demonstrated a robust increase in oxidative stress via multiple biomarkers at 376 µg/ml and when a surfactant is added, at 180.2 µg/ml. In HaCaT cells, a glyphosate formulation demonstrated significant increases in oxidative stress from doses starting as low as 1.7 µg/ml in a concentration-dependent fashion. No studies were available in human lymphocytes.

In Swiss CD-1 mice, a glyphosate formulation significantly increased oxidative stress in the kidney but only demonstrated a mild (non-significant) increase in the liver. This study evaluated oxidative stress at two different time points following exposure and saw responses that differed over time. The strong increase in the liver for glyphosate but not glyphosate formulation, suggests a complicated response pattern for pure glyphosate versus the formulation that could be linked to the time since exposure. In Swiss Albino mice, a glyphosate formulation demonstrated increased oxidative stress by two separate biomarkers in both the liver and the kidney. In a second study in Swiss albino mice using a different biomarker but a similar dose, increased oxidative stress was seen in both the liver and the kidney. In Wistar rat pups exposed in utero, an increase in oxidative stress was seen in the hippocampus and, in second study, in brain. In male Wistar rats, oxidative stress was clearly increased in the testis. In Swiss albino mice, topical application of a glyphosate formulation to the skin resulted in a proteomic fingerprint suggesting oxidative stress was increased. In Sprague-Dawley rats,

glyphosate increased oxidative stress in the kidney, liver and blood.

Though there are fewer studies for oxidative stress than there are for genotoxicity, the robust response seen here in human cells and in rodent studies clearly supports a role for both glyphosate and glyphosate formulations in inducing oxidative stress. Thus, there is a second reasonable mechanism through which the tumors seen in humans and those seen in animals can be caused by glyphosate and glyphosate formulations.

7.3 Summary for Biological Plausibility

In the evaluation of causality, the evidence for biological plausibility is overwhelming. Glyphosate clearly causes multiple cancers in mice, two cancers in the hematopoietic system similar to what is seen in humans, causes cancer in rats, is genotoxic and induces oxidative stress. The findings are clear for both glyphosate alone and for glyphosate formulations. **There is strong support for biological plausibility in support of a causal association of glyphosate and glyphosate formulations with NHL.**

8 Biological Gradient

A biological gradient exists for both the epidemiological data and the animal carcinogenicity data, thus there is support for a biological gradient.

Five of the epidemiological studies provided information on biological gradients in their publications.

Eriksson et al. (2008)[15] and **McDuffie et al. (2001)**[16] had consistent results for intensity of exposure per year (≤2 days per year, OR=1.0; ≤10 days per year, OR=1.69; >2 days per year, OR=2.12; >10 days per year, OR=2.26). **Pahwa et al. (2019)** [19] combined two studies and saw an increase for less that 3.5 years of exposure (OR 1.59 unadj., 1.28 adj.) and this dropped for >3.5 years of exposure (1.20 unadj., 0.94 adj.). Breaking exposure at 2 days per year there was clear dose-response (≤2, OR 1.03 unadj', 0.74 adj.; >2 days 2.42 unadj., 1.73 adj.). Both studies from the AHS [20, 21] showed no dose-response for any of their exposure metrics. However, the high frequency of exposure to many pesticides means subjects with low exposure to glyphosate were likely to be exposed to other agents that may also induce NHL; this could reduce the RRs in the higher exposure classes because it would inflate the RR in the low-exposure referent group. Also, problems previously mentioned regarding the exposure metric in Andreotti et al. (2018) [21] are likely to have contributed to the failure to see an exposure-response relationship.

It is not possible to resolve the differences between these five studies nor is it easy to argue that one study has more weight on this question than any other. The studies use different measures of exposure or time since exposure, are done on different populations and have different statistical power to detect a trend.

In rodent carcinogenicity studies, there is clear evidence of a biological gradient.

In general, there is support that a biological gradient exists for the epidemiological data and thus support from this aspect of the Bradford-Hill evaluation.

9 Temporal Relationship

Exposure must come before the cancers occur otherwise the epidemiology studies are useless. In this case, it is clear that exposure came before the onset of NHL. **The need for a temporal relationship in the data supporting a causal association between glyphosate and NHL is satisfied.**

10 Specificity

There are other causes of NHL[76-79] so this group of cancers is not specific to glyphosate. Glyphosate has been studied in multiple population for multiple types of tumors; the only class of cancers consistently seen as positive for exposure to glyphosate is NHL. **There is strong support for specificity**.

11 Coherence

There is strong support for coherence in the data supporting a causal association of glyphosate and glyphosate formulations with NHL.

Humans, coming into contact with glyphosate in multiple ways, can absorb the compound into their bodies where it has been measured in blood and in urine[5, 318-330]. In laboratory animals, and to some degree in humans, absorption, distribution and elimination of glyphosate and glyphosate compounds have been studied[231, 331-333] and show that glyphosate gets into the mammals' bodies, distributes to numerous organs and is eliminated in urine. The animal cancer studies clearly demonstrate that glyphosate in mammals can have toxic effects.

Mouse models have long served as surrogates for humans in understanding and developing treatments for many diseases. The same holds true for lymphoid tumors seen in humans. For over 30 years, mouse models have been studied and evaluated as surrogates for NHL[80-84]. These publications and the associated classification systems for humans and mice indicate a close linkage between the diseases in humans and mice. Thus, coherence is supported by the increased risk of malignant lymphomas in CD-1 mice, the marginal increase in these tumors in Swiss mice and the strong similarity between malignant lymphomas in mice and NHL in humans.

Wang et al. (2019) [85] exposed male and female Vk*MYC mice from the C57Bl/6 genetic background to glyphosate (purity not provided) at an exposure of 1 g/L in drinking water for 72 weeks (approximately 18 months) with an appropriate control. In addition, using the same mice, 7-day exposures were given at doses of 0, 1, 5, 10 and 30 g/L of glyphosate (n=5 per group). From the paper, "*A hallmark of multiple myeloma (MM) is that virtually all MM cases are preceded by monoclonal gammopathy of undetermined significance (MGUS). Bergsagel and colleagues [334] generated a mouse model of MM (Vk*MYC) under the C57bl/6 genetic background with sporadic c-Myc activation in germinal center B cells, resulting in the development of benign monoclonal gammopathy, a mouse equivalent to MGUS, which then progresses to MM. This is the best available MM animal model because it recapitulates many biological and clinical*

features of human MM, including increased serum immunoglobulin G (IgG), bone lesions, and kidney damage." Basically, this is a mouse that is genetically prone to get MM by a mechanism assumed to be the major pathway for MM in humans. All mice were subject to analyses for total serum IgG levels, blood cell counts, and total serum creatinine. Animals in the acute study and those surviving to 72 weeks (n=3) were also analyzed for M-spike detection (a method to diagnose MM) and pathology.

Glyphosate significantly (p not given) reduced survival in Vk* MYC mice after 72 weeks of exposure relative to the glyphosate-free controls. The treated mice had significantly increased spleen weight ($p<0.05$) and splenocyte counts ($p<0.05$). The treated mice also demonstrated histological disorganization and overall toxicity. Wild-type (WT) littermates (they contain one copy of the Vk*MYC gene rather than two) also demonstrated toxicity and disorganization, but to a lesser degree. Thus, glyphosate induced splenomegaly in both WT and Vk*MYC mice. Both WT and Vk*MYC mice demonstrated a significant increase ($p<0.05$) in IgG levels when compared to controls. Vk*MYC treated mice had a clear M-spike, WT mice had a weaker M-spike and no M-spike was detected in untreated animals regardless of genetics. In addition, there were multiple hematological abnormalities in treated versus untreated mice that were consistent with MM. There was also an increase in serum creatinine indicating kidney dysfunction. Pathological analysis of the kidney confirmed the damage in tubular cells and demonstrated tissue damage to the liver, lung and bone.

Activation-induced cytidine deaminase (AID, a marker of MGUS induction) was upregulated in both bone marrow and spleen of both Vk*MYC and WT mice in the 72-week study. The same upregulation in the spleen and bone marrow were seen in the 7-day exposure animals in a dose-dependent fashion with significant pairwise increases at 10 and 30 g/L. A smaller dose-dependent increase was seen in lymph nodes but this was still significantly different from controls at the highest exposure 30g/L. This upregulation of AID support an AID-mediated mutational mechanism for the induction of MM in these mice.

They conclude by noting that "*Our data disclose, for the first time, that glyphosate elicits a B cell-specific mutational mechanism of action in promoting carcinogenesis, as well as offering experimental evidence to support the epidemiologic finding regarding its tissue specificity in carcinogenesis (i.e., only increasing the risk for MM and NHL)*." Combined with the findings of malignant lymphomas in male mice, this study provides strong support that glyphosate causes NHL in humans and probably also causes multiple myeloma.

There is strong support for coherence in the data supporting a causal association of glyphosate and glyphosate formulations with NHL.

12 Experimental Evidence in Humans

There is no experimental evidence in humans since purposely exposing humans to a pesticide, especially one that is probably carcinogenic, is not ethical and would never pass review by a human subject's advisory board.

13 Analogy

I am unaware of any analogous compounds from the scientific literature. This, however, is not an area where I have sufficient background to express an opinion.

14 Summary of Bradford Hill Evaluation

Glyphosate probably causes NHL and, given the human, animal and experimental evidence, I assert that, to a reasonable degree of scientific certainty, the probability that glyphosate causes NHL is high.

Table 22 summarizes the information for each of Hill's aspects of causality. For these data, causality is strengthened because the available epidemiological studies show a consistent positive association between cancer and the exposure. The studies do not show different responses with some studies being positive and others negative, nor do they show any heterogeneity when analyzed together. And, in answer to Hill's question, the relationship between NHL and glyphosate exposure has been observed by different persons, in different places, circumstances, and times.

Causality is strengthened for these data because the strength of the observed associations, when evaluated simultaneously, are statistically significant, the findings are uni-directional and the results are unlikely to be due to chance. Even though none of the individual studies provide relative risks or odds ratios that are large and precise, the meta-analysis has objectively shown that the observed association across these studies is significant and supports a positive association between NHL and glyphosate.

Biological plausibility is strongly supported by the animal carcinogenicity data and the mechanistic data on genotoxicity and oxidative stress. When addressing biological plausibility, the first question generally asked is "Can you show that glyphosate causes cancers in experimental animals?" In this case, the answer to that question is clearly yes. Glyphosate has been demonstrated to cause cancer in two strains of rats and one strain of mice. Glyphosate has been demonstrated to cause cancer in two strains of rats and one strain of mice. Glyphosate causes hepatocellular adenomas in male Wistar rats and, to a lesser degree, in male Sprague-Dawley rats, mammary gland adenomas and adenocarcinomas in female Wistar rats, skin keratocanthomas in male Wistar rats, and kidney adenomas and thyroid C-cell adenomas and carcinomas in male Sprague-Dawley rats. Glyphosate causes hemangiosarcomas, kidney tumors and malignant lymphomas in male CD-1 mice and hemangiomas in female CD-1 mice and possibly causes malignant lymphomas in male Swiss albino mice. Thus, it is biologically plausible that glyphosate alone can cause cancer in mammals.

The next question generally asked is "Does the mechanism by which glyphosate causes cancer in experimental animals also work in humans?" The best understood mechanism by which chemicals cause cancer in both humans and animals is through damaging DNA that leads to mutations in cells that then leads to uncontrolled cellular replication and eventually cancer. It is absolutely clear from the available scientific data that both

glyphosate and glyphosate formulations are genotoxic. This has been amply demonstrated in humans that were exposed to glyphosate, in human cells *in vitro*, in experimental animal models and their cells *in vitro* and *in vivo*, and in wildlife. One way in which DNA can be damaged is through the presence of free oxygen radicals that overwhelm a cell's antioxidant defenses. Glyphosate induces this type of oxidative stress, providing additional support for a biological mechanism that works in humans.

Table 22: Summary conclusions for Hill's nine aspects of epidemiological data and related science

Aspect	Conclusion	Reason
Consistency of the observed association	Strong	Multiple studies, most are positive, meta-analysis shows little heterogeneity and positive findings, different research teams, different continents, different questionnaires, no obvious bias or confounding
Strength of the observed association	Strong	Seven core epidemiology studies all show the same modest increase, significant meta-analyses
Biological plausibility	Very Strong	Multiple cancers in multiple species, not due to chance, increased risk of rare tumors, convincing evidence for genotoxicity and oxidative stress
Biological gradient	Moderate	Clearly seen in the two case-control studies and a pooled analysis that evaluated it, not seen in the cohort study
Temporal relationship of the observed association	Satisfied	Exposure clearly came before cancers
Specificity of the observed association	Strong	The only cancers linked to glyphosate are NHL and its subtypes
Coherence	Strong	Glyphosate is absorbed, distributed and excreted from the body, cancers seen in the mice have strong similarity to human NHL and transgenic animals support a brifge between findings in the humans and the mice
Evidence from human experimentation	No data	No studies are available
Analogy	No data	No studies available in the literature

In general, there is support that a biological gradient exists for the epidemiological data and thus support from this aspect of the Bradford-Hill evaluation. Glyphosate ORs increased with time since first exposure and with intensity of use per year in the three related case-control studies that evaluated at least one of these issues.

There is clearly the proper temporal relationship with the exposure coming before the

cancers.

The human evidence is coherent. The basic findings in humans agree with the animal evidence for absorption, distribution and elimination of glyphosate. Also, one of the tumors seen in mice has almost the same etiology as NHL and transgenic animals prone to get B-cell tumors through a mechanism linked to humans show increased risk after exposure to glyphosate.

NHL is not specific to glyphosate exposure however, glyphosate is specific to NHL. There is no experimental evidence in humans and I did not find any references where researchers looked for analogous compounds with similar toxicity.

Hill (1965)[4] asks "*is there any other way of explaining the set of facts before us, is there any other answer equally, or more, likely than cause and effect?*" There is no better way of explaining the scientific evidence relating glyphosate to an increase in NHL in humans than cause and effect.

In my opinion, glyphosate probably causes NHL and, given the human, animal and experimental evidence, I assert that, to a reasonable degree of scientific certainty, the probability that glyphosate causes NHL is high.

15 The IARC Assessment of Glyphosate

In March 2015, the International Agency for Research on Cancer (an agency of the World Health Organization) brought together seventeen scientists (the Working Group) to evaluate the scientific evidence on whether glyphosate can cause cancer in humans. This group also contained one invited specialist (myself) to aid the Working Group (WG) in going through the science but who was not allowed to join discussions on the final conclusion or write any part of the document. The Working Group concluded that glyphosate falls in the category *"probably carcinogenic to humans (Group 2A)"*[5].

The IARC preamble[6] guides Working Groups on how to evaluate scientific literature to determine if something is a hazard. All Working Groups follow these guidelines and this process is accepted worldwide as a proper way to evaluate the literature for a hazard (e.g., the European Chemical Agency cites the IARC review process as guidance and then uses the exact same wording as IARC does to guide their own hazard evaluation process[1]).

The WG examined the epidemiological data and classified it as "*limited evidence of carcinogenicity,*" which is defined to mean "*a positive association has been observed between exposure to the agent and cancer for which a causal interpretation is considered to be credible, but chance, bias or confounding could not be ruled out with reasonable confidence*." This is a precise and clear description of the strength of the evidence from the epidemiological studies.

The WG examined the evidence from animal carcinogenicity studies and classified it as "*sufficient evidence of carcinogenicity,*" which IARC defines as: "*a causal relationship has been established between the agent and an increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in (a) two or more*

species of animals or (b) two or more independent studies in one species carried out at different times or in different laboratories or under different protocols. A single study in one species and sex might be considered to provide sufficient evidence of carcinogenicity when malignant neoplasms occur to an unusual degree with regard to incidence, site, type of tumour or age at onset, or when there are strong findings of tumours at multiple sites." Based on the data available to IARC at the time of their review and the restrictions placed on the studies they can review by the Preamble, this conclusion is justified and correct.

One of the major criticisms of the WG review was that the WG did not review all of the animal carcinogenicity data that was available to the regulatory bodies and thus came to the wrong conclusions on the animal cancer data. In this review, I evaluated all 23 animal carcinogenicity experiments that have been collectively mentioned by any agency that reviews glyphosate. Where possible, I have analyzed the original data and used sound statistical methods to test for significant increases in cancer incidence in animals exposed to glyphosate. My conclusion is that the WG would have called this data "*sufficient evidence*" to support their findings despite not reviewing the additional studies analyzed herein. Despite the fact the industry kept these studies confidential, nothing contained in the withheld studies would have changed the WG conclusion.

On the mechanistic data, the IARC Working Group reviewed the same data that I reviewed up to 2015, but I also evaluated, where possible, the proprietary data supporting the regulatory decisions and newer published data. Where possible, I reanalyzed that data to be certain the results being presented were accurate. The IARC Working Group, using the guidelines set forth in their Preamble, declared strong support for the biological mechanisms of genotoxicity and oxidative stress. As I have shown here, there is strong support for these two mechanisms, even with the proprietary evidence from the industry studies. Thus, the IARC Working Group reached the correct conclusion.

To decide on a final classification for a compound, the IARC Preamble provides guidance on how the classification of the three areas are to be used. If the data in humans is "*limited*" and the data from animal carcinogenicity studies is "*sufficient*," the discussions should begin with Class 2A, "*the agent is probably carcinogenic to humans*." Then, given the overall quality of the data set, the strength of the evidence from the mechanistic studies and any additional scientific issues that need to be considered, the Working Group will determine whether the data justifies a different category. In this case, the Working Group concluded 2A was the right category and I still believe the evidence supports that finding.

16 Regulatory Assessments of Glyphosate

Like IARC, the EPA has guidelines that are to be followed when evaluating scientific literature and making a determination about the carcinogenic potential of a chemical. Those guidelines have been developed over many years and are based on sound scientific guidance that myself and many other scientists have provided to the Agency. For their evaluation of glyphosate, the Agency did not follow their own guidelines, nor

did they follow sound scientific practice. This opinion is consistent with the review done by the **EPA FIFRA Scientific Advisory Panel**[121]. In addition, the Agency failed to find all of the relevant animal cancer studies and misinterpreted several of them. The major problems with the Agency evaluation are:

- Misinterpretation of the epidemiological evidence, confusing the potential for bias and potential for confounding with real bias and real confounding, allowing them to give almost no weight to the case-control studies in favor of the one cohort study;
- Misinterpretation of the findings in the meta-analysis;
- Failure to properly use historical controls in the analysis of the animal carcinogenicity studies; declaring a significant finding as not due to the compound if it is in the range of the historical controls;
- Failure to analyze all tumors in all studies relying upon the industry submissions to have done this correctly;
- Failure to follow their guidelines on what constitutes a positive finding, disregarding significant trend tests when no corresponding pairwise comparisons are also significant;
- Disregarding positive findings in doses that are clearly not above the maximum dose the animals could be given without compromising the integrity of the study;
- Using unreasonable arguments about the overall false positive rates in the study without actually doing an analysis of this issue;
- Failing to recognize the similar findings in similar studies and to do a pooled analysis to determine if the negative effects in one study cancel out the positive effects in another;
- Giving very little weight to studies from the literature and relying almost entirely on studies provided by industry that have not undergone peer review for both quality and, more importantly in some cases, interpretation of the findings; and
- Comparing results across different species and strains for the animal cancer studies and the mechanistic studies with little regard for unique findings in any one study and consistent findings across multiple studies.

Similar comments apply to evaluations done by the **European Food Safety Authority**[55], the **European Chemical Agency**[335], the **Australian Pesticides and Veterinary Medicines** [336-338], the **New Zealand Environmental Protection Agency** [339] and the **Canadian Pest Management Regulatory Agency** [340]. My detailed comments to these agencies on their risk assessments are publicly available. There were comments to my comments to EPA by other scientists and I also responded to those comments in the EPA docket for glyphosate.

There is one issue worth spending a bit more time on. In 2010, the Canadian Pest

Management Regulatory Agency (PMRA) published a re-evaluation work plan for glyphosate outlining the focus of the re-evaluation and indicating that the PMRA planned to work cooperatively with the United States Environmental Protection Agency (USEPA) on the re-evaluation of glyphosate. In 2015, PMRA published a preliminary re-evaluation (PRVD) for glyphosate [341], allowed public comments on this document then published a final re-evaluation (RVD) in 2017 [340]. They then published a "Notice of Objections" [342] in response to a variety of objections to the methods and scientific evaluations done by the PMRA in the PRVD [341] and the RVD [340]. The decision that is being taken has to do with the establishment of a review panel based on the validity and scientific plausibility of the issues raised by the petitioners. They concluded the information does not meet the factors needed to establish a review panel and they dismiss the concerns of the petitioners. Many of the comments pertain to issues other than the carcinogenicity of glyphosate and are outside the scope of my review. However, one pertains to carcinogenicity and a note I wrote to the President of the EU. Here is their summarization of the comment.

Comment 1: A comment was received which contained the most recent open letter (May 2017) from Dr. Christopher Portier, Chair of the IARC Committee, to Jean-Claude Juncker, President of the European Commission, concerning the European Food Safety Authority's (EFSA's) re-evaluation of glyphosate. The open letter states that, following an evaluation of the raw tumour data from the EFSA re-evaluation, Dr. Portier believes that the evaluations applied to the glyphosate data are scientifically flawed (specifically as it pertained to statistical analysis), and any decisions derived from these evaluations will fail to protect public health.

In their response, the PMRA notes that EFSA and others responded to my letter to President Juncker and that they agree with the response provided by these European agencies (EA). Hence, my response will focus on the response sent to me by the EA.

The letter [154] sent to President Juncker provided an executive summary as follows:

> "*The European Food Safety Agency IEFSA) and the European Chemical Agency IEChA) have completed their assessments of the carcinogenic potential of glyphosate and concluded that the evidence does not support a classification for glyphosate. The raw data for the animal cancer studies for glyphosate have been released, and a reanalysis of these data show eight instances where significant increases in tumor response following glyphosate exposure were not included in the assessment by either EFSA or EChA. This suggests that the evaluations applied to the glyphosate data are scientifically flawed, and any decisions derived from these evaluations will fail to protect public health. I ask that the evaluations by both EFSA and EChA be repeated for all toxicological endpoints and the data underlying these evaluations be publicly released.*"

In essence, there were eight additional tumor sites in the animal carcinogenicity data that were not cited or mentioned in the evaluation done in the European Food Safety Agency (EFSA) and German Federal Institute for Risk Assessment (BfR) reviews of glyphosate. In addition, the letter summarized concerns in a previous letter [122] sent by my colleagues and I to Commissioner Andriukaitis regarding the EFSA/BfR reviews of

glyphosate. The letter also summarized all of the reasons cited by EFSA/BfR for excluding tumor findings they did mention/evaluate. The letter to President Juncker raises concerns about the other areas of review (e.g. reproductive toxicity, endocrine disruption) and suggests they may have also received inadequate evaluations. I recommend that these studies be completely re-analyzed by EFSA, that all of the data be released for independent scientists to evaluate as well, and that a new risk assessment for glyphosate be produced with all positive findings discussed in detail.

The summary of the major concerns raised in the original letter [122] that were not adequately addressed in the final assessments are as follows:

- the classification of the human evidence as "very limited" is not a valid characterization under the CLP guidelines and fails to properly address the strength of the available evidence;

- both EFSA and EChA dismissed positive findings because they fell inside of the range of the historical controls (this is an improper use of historical control evidence);

- both EFSA and EChA compared findings across different strains and different study durations to conclude that studies were inconsistent (this is not scientifically justifiable);

- both EFSA and EChA characterize the evidence for genotoxicity as negative, yet a careful review of the evidence released by EFSA and the open scientific literature suggest there are many guideline and non-guideline studies demonstrating genotoxicity.

The response from EFSA/EChA [343] contained a cover letter and a more detailed technical response. The cover letter concluded:

> "*Overall EFSA and ECHA are of the opinion that all the findings on the chronic rodent carcinogenicity studies referred to in your letter have been adequately considered and therefore we see no need for out evaluations to be revisited.*"

The detailed technical response covers their reasons for reaching this conclusion. The technical response begins with a description of their process, a statement that they have answered all my previous comments and will not readdress them and then made several assumptions about my analyses that were correct with the exception that I analyzed all of the data; as for this report, I restricted the analyses to sites with 3 or more tumors and I only presented those with a statistically significant positive trend. They discuss their key guidance document, **Guidance on the Application of the CLP Criteria: Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures** [1].

The EA letter then goes on to discuss their philosophy for the review of the experiments. One principle they use is part of an OECD guidance [28] that states "*The central concept*

of this document is that the experimental design represents the strategy for answering the question of interest and that the specific statistical analyses are tactical methods used to help answer the questions. Therefore, the statistical methods most appropriate for the analysis of the data collected should be established at the time of designing the experiment and before the study starts." This advice is flawed for animal carcinogenicity studies where the experimental design is effectively codified. The best test to use for these studies is a trend test, like the one used in this report, as explained in Section 7.1.1. They do agree that re-analysis is also a reasonable approach and that, if spelled out in advance, would avoid biasing the analysis. What is ignored is that by allowing the regulated industry to do any test they wish, they will choose the method of analysis least likely to show a positive result.

The EA letter then goes on to discuss one-sided versus two-sided tests in the abstract; they never discuss which is more appropriate in a review of safety for a chemical. This was the point of my comments on this subject; if the purpose is to evaluate whether something can cause harm, the alternative hypothesis in the statistical test should be it causes harm and the two-sided alternative hypothesis that is causes harm or is protective is illogical.

Next, the EA letter provides some discussion of correction for intercurrent mortality and exceedance of the MTD. However, as noted by both EFSA and ECHA in their own reviews, there is no intercurrent mortality in any of these studies and no study appears to have exceeded the MTD. This is followed by an abstract paragraph about trend tests versus pairwise comparisons that provides no response to any of my concerns. This is then followed by a discussion of the question of multiple testing and the overall rate of false-positives which I have addressed directly in Section 7.1.6.

After this, the EA letter finally addresses the 8 additional tumors that had not been addressed in the original review. They discuss the difference between statistical significance and biological significance, a difference I do not dispute and cite the IARC Preamble [6], of which I am a co-author, as justification. Finally, they note that "*It is acknowledged that 7 out of 8 tumour findings reported by Dr Portier were not specifically documented either in the RAR or CLH report. As Indicated below, the reason for that was not that they would have been overlooked or dismissed but they were considered not relevant for hazard and risk assessment.*" They then went on to discuss each tumor finding, something that should have been done in the original document.

The first finding they list is the increase in adenocarcinomas of the lung in CD-1 mice in the study by **Wood et al. (2009)** [51]. They note that this finding was discussed in the original study report provided to them by the Glyphosate Task Force. In the industry report, they write "*Lung turnouts of alveolar/bronchiolar cell origin, both adenomas and adenocarcinomas are commonly encountered in the aging CD-1 mouse and such was the case in this study.*" They also said the following "*For male mice, slightly more adenocarcinomas were diagnosed among high dose animals compared to controls but the potential for progression from benign bronchiolar/alveolar neoplasms of the lung to malignant forms for this type of neoplasm makes an assessment of the combined incidence of adenomas and adenocarcinomas more reliable (Brooks and Kellington,*

1998)." In their response to me, the EFSA/ECHA write:

"*Lung turnouts of alveolar/bronchiolar cell origin, both adenomas and adenocarcinomas are commonly encountered in the aging CD-i mouse and this was the case in this study. For male mice, slightly more adenocarcinomas were diagnosed among high dose animals compared to controls but the potential for progression from benign bronchiolar/alveolar neoplasms of the lung to malignant forms for this type of neoplasm makes an assessment of the combined incidence of adenomas and adenocarcinomas more reliable. If this approach is taken, there was no evidence of an increase.*"

Thus, their answer is almost verbatim what industry wrote in the original report. In the industry report, there were no statistical evaluations provided. What has happened in this case is that the number of adenomas (benign tumors) in the lung have dropped and the number of adenocarcinomas (malignant tumors) has increased as a function of dose (see Table 14). A chemical that causes a progression from benign neoplasms to malignant neoplasms is known as a promoter and cannot be dismissed as non-carcinogenic. The **CLP guidelines** [1], define a chemical to exhibit "**limited evidence of carcinogenicity**" to chemicals where "*the evidence of carcinogenicity is restricted to studies that demonstrate only promoting activity*", it does not dismiss this finding as negative.

The next tumor was hemangiomas in female CD-1 mice in the study by **Sugimoto et al. (1997)** [50]. The EA states "*As explained in the weight of evidence assessment, an increased incidence of benign tumours observed only at an extremely high dose (exceeding 4000 mg/kg bw per day) well above the MTD is less relevant for classification; even if they are not automatically excluded from any consideration.*" And "*It is important to note that no progression to malignant haemangiosarcoma was observed.*" The actual tumors observed (see Table 13) were in both the mid-dose group and the high-dose group with a clear statistical trend in the data (p_{Trend}=0.002). Going back to the CLP Guidelines, it states that a chemical has "***limited evidence of carcinogenicity***" if "*the agent increases the incidence only of benign neoplasms or lesions of uncertain neoplastic potential*"; the guidance does not dismiss these findings. Finally, hemangiomas do not progress to hemangiosarcomas.

Thyroid follicular cell adenomas in male Sprague-Dawley rats in **Atkinson et al. (1993)** [30] was the next tumor they discussed. Even though they mischaracterize the evidence, I find this tumor to be equivocal evidence and will not discuss it further.

Thyroid C-cell carcinomas in female Sprague-Dawley rats from **Lankas (1981)** [137]was next. They initially discuss the low exposures from this study and state that "*Thus, one would not expect effects at such a low dose as the maximum one in the study by Lankas.*" In essence, they dismiss a result because it is positive at a low dose which is the opposite of what they have usually done by dismissing studies with positive findings at high doses. They correctly noted there was no increase in hyperplasias in thyroid C-cells in any dose group but failed to note that study B also showed an increase in thyroid c-cell adenomas in female Sprague-Dawley rats. They also failed to discuss the historical control data provided by the study author which showed a range of 0% to 5.2% in 9

control groups so the observed response at the highest dose (12.8%) was outside of the range of the historical controls and very significant using a proper statistical test (p_{Hist}<0.001).

The study by **Enemoto (1997)** [34] saw an increase in kidney adenomas in male Sprague-Dawley rats. The EA response states "*The incidence of kidney adenomas at the high dose (4/50) compared to the control was discussed in the study report. It was stated to be above the background incidence of this tumour in the strain of rat (0.7% - range 0.0-2.9%), but was not statistically significant in the pairwise comparison. The absence of pre-neoplastic renal changes, the fact that the four tumours were observed at a dose of 30000 ppm (Ca. 1127 mg/kg bw per day in males) that was considered to exceed the Maximum Tolerated Dose (MTD) as evidenced by reduced body weight, body weight gain and food efficiency, and gastro intestinal effects and the lack of progression towards malignancy support the conclusion that they were not relevant for hazard and risk assessment.*" Not once in the original EFSA review do they state the MTD was exceeded. This is not surprising since the OECD definition of the MTD is "*The concept of the Maximum Tolerated Dose (MTD), conventionally defined as the highest dose to produce toxic effects without causing death and to decrease body weight gain by no more than 10% relative to controls (OECD 2002, GD No. 35) became well established.*"; the reduction seen in body weights in this study was 7%. The pooled analysis was positive in male Sprague-Dawley rats. Despite their discussion at the beginning of not relying so heavily on statistical significance, they exclude this finding because the pairwise comparison was p_{Fisher}=0.059. Thus, we have a rare tumor, outside the range of historical controls with a fairly strong difference from controls and an increase in kidney tumors in CD-1 mice yet they discard this finding as not relevant.

Brammer (2001) [31] saw an increase in liver adenomas in male Wistar rats not examined in the ECHA and EFSA reviews. They note the tumors were discussed in the original industry report and then provide an almost verbatim repeat of what was in the industry report. They note there were no preneoplastic lesions for this tumor in this study. What they fail to note is the increases seen in Sprague-Dawley rats and the preneoplastic foci seen in studies with other Wistar rats. In addition, the pooled analyses in Wistar rats and the pooled analyses in Sprague-Dawley rats for liver adenomas are both statistically significant.

The finding of an increase incidence of skin keratoacanthomas in male Wistar rats in **Wood et al. (2009)** [42] was dismissed in the EA letter because all skin tumors combined was not positive and because the pair-wise comparison by Fisher's exact test was not positive. Again, they fail to mention the increases in these tumors in male Sprague-Dawley rats and they failed to do a pooled analysis indicating all three studies in Wistar rats, when combined, support a positive finding.

Finally, also in **Wood et al. (2009)**, there was an increase in mammary gland adenomas and adenocarcinomas in female Wistar rats. Again, the answer provided in the EA letter is almost verbatim what was written in the original study report. There is no indication that ECHA or EFSA went beyond the study report to examine these data in any great degree. Given the findings in the study by **Seralini et al. (2014)** [39], this finding should

have been provided in the original review and discussed in detail.

The remaining text in the EA letter is a discussion, after the fact, of why the EChA and EFSA knew about all of these tumors and why they do not matter to the overall conclusions. They reject the notion that their reviews were inadequate. I still disagree with this conclusion. Many of these tumors were also not mentioned in the EPA review.

Dr. Christopher J. Portier

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