# EXHIBIT 94

## Expert Report
## Christopher J. Portier, Ph.D.

## 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.

## 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[1, 2]; 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[3, 4]; 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[5, 6]. 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[7-19].  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[20-24] 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[25]. 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[26-28].

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[29].

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)[30].  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.

## Reliance List

During the course of my preparation for this report, I have reviewed the following materials:

   a. All epidemiological data relating to the ability of glyphosate formulations to cause NHL in humans.

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b.  Scientific papers on the cellular origins of NHL
c.  Peer-reviewed scientific data relating to the carcinogenicity, genotoxicity and oxidative stress caused by glyphosate
d.  Technical reports relating to the carcinogenicity of glyphosate provided by the defendant to the lawyers for the plaintiff
e.  The USEPA, the European Food Safety Authority (EFSA), the German Federal Institute for Risk Assessment, the European Chemical Agency, the IARC and the WHO/Food and Agriculture Organization Joint Meeting on Pesticide Residues reviews of the scientific literature relating to the potential for glyphosate to cause cancer.
f.  Technical documents available from EFSA regarding animal carcinogenicity data on glyphosate prepared by organizations other than the defendant
g.  Various other documents produced in the litigation

A complete list of my reliance materials is at the end of this report.

## Methodology for Causality Evaluation

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[31, 32] 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[30, 33-35] that rely upon aspects of the criteria for causality developed by **Hill (1965)**[36].

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

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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[37], 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. This issue is seldom applicable and, since NHL has other causes, specificity is not applicable to the determination of causality for glyphosate.

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.  No such data are available for glyphosate.

The most logical approach to developing an inference of causality is to step through each of the aspects of causality developed **by Hill (1965**)[36] and apply them to the available data for glyphosate and for glyphosate formulations.  This is done in the sections that follow.

## Consistency of the Associations seen in Human Epidemiological Studies

### Relevant Epidemiology Studies

In their meta-analysis, **Chang and Delzell (2016)**[38] 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[39-50].  Their search agrees with all current reviews of glyphosate and I will use their findings from the literature up until 2015.  To cover from June 2015 to the present (April 1, 2017), I used their searching algorithm and identified 117 additional published studies, none of which were new epidemiology studies.  These same 12 studies will be considered for use in this evaluation.  Other experts will be discussing the studies as well as their strengths and their weaknesses; I will focus on using the results of these studies in evaluating causality so I will only briefly describe each study.

**Cantor et al. (1992)**[39] 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

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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[1] (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[2].  However, the authors note that the bias could both increase or decrease the OR because of non-differential exposure misclassification[3] 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)**[43]

Two additional studies conducted by **Zahm et al. (1990)**[51] in Nebraska and **Hoar et al. (1986)**[52] in Kansas collected information on pesticide and herbicide use, but did not report specifically on the effects of glyphosate.  **De Roos et al. (2003)**[43] pooled the data from these two studies with the data from **Cantor et al. (1992)**[39] to examine pesticide exposure to glyphosate in farming as risk factors for NHL.  The three case-control studies[39, 51, 52] had slightly different designs.  The design for the Minnesota study[39] is

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[1] 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.

[2] 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.

[3] 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.

provided directly above.  In Nebraska[51], 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[52], 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 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[52], 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 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 is the strongest study with sufficient power (3.8% of subjects exposed) and will be included in the evaluation of causation.

**Lee et al. (2004)**[44] pooled data from **Zahm et al. (1990)**[51] and **Cantor et al. (1992)**[39] (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)**[43] 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)**[43] with the exception of the inclusion of the few women in the study by **Zahm et al. (1990)**[51].  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)**[40] 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% (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[42] 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)**[41] 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

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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[42] 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)**[42] conducted a pooled analysis of NHL and HCL by combining the studies of **Nordstrom et al. (1998)**[40] and **Hardell and Eriksson (1999)**[41]. 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)**[46] 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)**[50] 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)**[52] and **Zahm et al. (1990)**[51] 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)**[48] re-analyzed the data of **McDuffie et al. (2001)**[50] 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

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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)**[38] 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)**[50], 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)**[47] 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)**[49] 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

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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.

**De Roos et al. (2005)**[45] 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[4] (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

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[4] 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).

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[45].

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[53]: 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)[54] in their review of the EPA's issue paper on the carcinogenicity of glyphosate and as noted in a critique[55] 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 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.

## Consistency of Associations

**Hill (1965)**[36] 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

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and half above one.  As noted by both the **IARC Monograph 112 (2015)**[56] and by **Chang and Delzell (2016)**[38], 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 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.
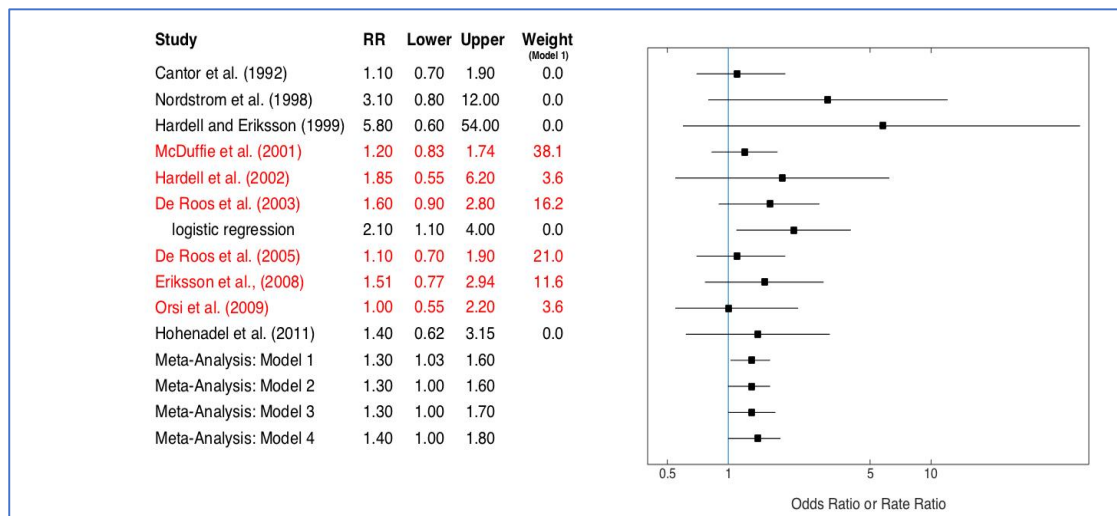
The first obvious conclusion to be drawn from Figure 1 is that all of the mean OR/RR estimates (black squares) are consistently ≥1.  This implies that all of the studies are pointing in the same direction toward a positive effect.  In their meta-analyses, **Schinasi and Leon (2014)**[57], **IARC (2015)**[56] and **Chang and Delzell (2016)**[38] all identified 6 papers (highlighted in red in Figure 1) as being the most reliable for evaluation of the ability for glyphosate to induce NHL in people: **McDuffie et al. (2001)**[50], **Hardell et al. (2002)**[42], **De Roos et al. (2003)**[43] and **(2005)**[45], **Eriksson et al. (2008)**[46] and **Orsi et al. (2009)**[47].  I will refer to these papers as the six 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 6 core studies, you can see that all are ≥1; the probability of this happening is $(0.5)^6$ or 0.016, 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 RR (CRR) and test for heterogeneity in the studies.  The meta-analysis done by **Chang and Delzell (2016)** includes the same analysis as that done by the **IARC (2015)** and is an improvement over **Schinasi and Leon (2014),** so I will focus my comments on using the **Chang and Delzell (2016)** meta-analysis.  **Chang and Delzell (2016)** did four separate meta-analyses on the glyphosate epidemiology studies using two different methods (random-effects and fixed-effects models).  In their first analysis (model 1)[5], they combined the most-fully-adjusted risk estimates from the six 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-

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[5] **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.

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 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, none of the different meta-analyses rejected the notion of a combined, statistically significant positive effect.

**Figure 1:** Odds Ratios and Rate Ratios from the most-fully-adjusted risk estimates from selected epidemiology studies and from the meta-analyses of **Chang and Delzell (2016)**[38]. "RR" refers to the OR or RR from the study, "Lower" refers to the 95% lower bound, "Upper" to the 95% upper bound and "Weight" refers to the weight applied to that specific study in Model 1 of the meta-analysis (Table 3 in Chang and Delzell). For **De Roos et al. (2003)**, the first row is for the Bayesian model analysis and the second row, labelled "logistic regression" is from the logistic model analysis.



| Study | RR | Lower | Upper | Weight (Model 1) |
|---|---|---|---|---|
| Cantor et al. (1992) | 1.10 | 0.70 | 1.90 | 0.0 |
| Nordstrom et al. (1998) | 3.10 | 0.80 | 12.00 | 0.0 |
| Hardell and Eriksson (1999) | 5.80 | 0.60 | 54.00 | 0.0 |
| McDuffie et al. (2001) | 1.20 | 0.83 | 1.74 | 38.1 |
| Hardell et al. (2002) | 1.85 | 0.55 | 6.20 | 3.6 |
| De Roos et al. (2003) | 1.60 | 0.90 | 2.80 | 16.2 |
| logistic regression | 2.10 | 1.10 | 4.00 | 0.0 |
| De Roos et al. (2005) | 1.10 | 0.70 | 1.90 | 21.0 |
| Eriksson et al., (2008) | 1.51 | 0.77 | 2.94 | 11.6 |
| Orsi et al. (2009) | 1.00 | 0.55 | 2.20 | 3.6 |
| Hohenadel et al. (2011) | 1.40 | 0.62 | 3.15 | 0.0 |
| Meta-Analysis: Model 1 | 1.30 | 1.03 | 1.60 | |
| Meta-Analysis: Model 2 | 1.30 | 1.00 | 1.60 | |
| Meta-Analysis: Model 3 | 1.30 | 1.00 | 1.70 | |
| Meta-Analysis: Model 4 | 1.40 | 1.00 | 1.80 | |

Odds Ratio or Rate Ratio

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[58].  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 of the meta-analyses as showing a positive association and strong consistency of the findings across the six core studies.

**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)**[46]with those of **Cocco et al. (2013)**[49] 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)**[46] with those of **Orsi et al. (2009)**[47] 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)**[46] with those of **Orsi et al. (2009)**[47] 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)**[46]with those of **Orsi et al. (2009)**[47] 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)**[40] with those of **Orsi et al. (2009)**[47] 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.

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

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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 six 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.

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.  Four[42, 43, 45, 46] of the six core studies controlled for exposure to other pesticides and saw basically the same findings as the other two studies.  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 six core epidemiology studies done on two different continents by four different research groups using different designs, questionnaires and study populations that are highly consistent with no obvious bias or confounding that would explain the results. **There is a consistency of associations across the six core studies.**

## Strength of the Association seen in Human Epidemiological 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 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)[45] 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)**[45], none of the core studies demonstrate large, precise risks as envisioned by **Hill (2016)**[36].  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 and Eriksson (1999)** 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 1 all demonstrate estimates of OR/RR that are significantly different from 1 rejecting the concept that the overall association is due to chance.  The statistically significant estimate of the OR/RR for B-cell lymphomas in the meta-analysis support this finding as well.

In summary, we have six 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 six core studies**

## Biological Plausibility

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.

### Animal Cancer Bioassays

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) 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[59]).  Other groups[30, 33, 34] 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[60].  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) or limit dose.  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[33, 59] on how to choose this top dose. These guidelines are in general agreement with the scientific literature[60].

The guidelines also address the methods by which the data should be analyzed.  For example, the EPA guidelines[61] 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[30, 59, 60, 62] 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[54]. The US National Toxicology Program (NTP) uses both a trend test[3, 4, 63] 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. In cases where the data is pooled and the numbers of tumors are large, the approximate p-value based upon the normal distribution is used for the trend test to avoid excessive computation time; these are noted as $p_{TrendA}$. The approximation ($p_{TrendA}$) is generally equivalent to the exact p-value ($p_{Trend}$) when there are more than 10 animals with tumors[64].

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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[54] 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. 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[30, 34, 59, 65, 66]. 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 the mean tumor response in the controls to calculate the probability of observing the trend seen in the study or a more significant trend if the true probability of response is the historical control average; 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[30, 33, 34, 67] 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[30]. 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[54] on the EPA issue paper on glyphosate[61] and with all guidelines for analyzing animal carcinogenicity data.

There are 13 animal carcinogenicity studies in rats[68-80] and eight in mice[81-88]. Only two studies[71, 77] appear in the peer-reviewed literature; the remaining studies are partially available through several sources. For three of the rat studies[70, 74, 78] and two mouse studies[83, 86], technical reports from the performing laboratory are available from documents provided by the registrant. For the remaining unpublished studies, data was obtained from the EPA review of glyphosate[61], the European Food Safety Authority review of glyphosate[89, 90] and supplemental material from a review of the carcinogenicity of glyphosate by a panel of scientists on behalf of Monsanto[91].

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.

It is unusual to have multiple carcinogenicity studies in the same experimental animal model arising from different laboratories. Methods for the combined analysis of multiple animal cancer bioassays are not available in the scientific literature. However, pooled analyses, as conducted in epidemiology[92, 93] are applicable for combining animal carcinogenicity studies. The basic concept is to pool all data from the same sex/species/strain into one study and analyze it appropriately. The basic steps are: 1) select the studies to be pooled; 2) merge the data for analysis; 3) estimate study specific

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effects; 4) estimate pooled effects; 5) explain the differences between the pooled effects and the individual study effects; 6) do a sensitivity analysis if possible.  These steps will be used to analyze pooled data from animal carcinogenicity studies where pooling is done by sex, species, strain and duration of exposure to limit heterogeneity across pooled studies.  In their recommendations to the EPA regarding EPA's issue paper on the carcinogenicity of glyphosate[54], the FIFRA Science Advisory panel strongly supported the use of a pooled analysis to address the question of consistency citing my comments to the EPA[94].

**Rat Studies**

**Reyna and Gordon (1974)[76]** exposed Albino rats (probably Sprague-Dawley) to ammonium salt of glyphosate (13.85% purity) in a two-year chronic feeding study.  Only EPA[61] 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.  Insufficient detail is available on this study.

This study is inadequate for use in deciding on causality.

**Burnett et al. (1979)[70]** 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[61] 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.  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)[74]** exposed groups of 50 male and 50 female Sprague-Dawley rats to glyphosate (98.7% purity) in feed (see Table 1 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[89], it was in general accordance with the 1981 OECD guidelines. Information on this study was available from EPA[61], EFSA[89], Greim et al.[91], the original study report from Bio/dynamics Inc.[95] and memos from Monsanto to EPA provided by Monsanto.

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

Table 1 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). Comparing this historical control mean to the observed response yields $p_{Hist}$=0.006, showing that this result is significant, even when comparing it to the historical control dataset. **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[33, 34, 59]; 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.  **EFSA**[89] noted rates for interstitial cell hyperplasia (a potential precursor for the interstitial cell tumors) and saw no dose-response trend (Table 1).  However, these 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"[54].

An increase in Thyroid C-cell carcinomas (Table 1) 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 mean responses of 4/453=0.9% for carcinomas and 46/453=10.2% for the combined tumors. The significance of both results was unchanged using the historical control data.

The authors also mentioned that the incidence of lymphocytic hyperplasia in the thymus and lymph nodes were slightly elevated above controls ($p_{Trend}$=0.143).  The middle dose group was significantly different from controls ($p_{Fisher}$=0.018).

This study also had a statistically significant increase in pancreatic islet cell tumors in the lowest dose ($p_{Fisher}$=0.028) in males (Table 1), but not any of the other doses; the trend test was not significant ($p_{Trend}$=0.312).

The highest dose used in this study in Sprague-Dawley rats is far below the MTD. Even though EFSA[89] 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[61] also excluded this study from consideration. 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 1.

**Table 1:** Tumors of interest in male and female Sprague-Dawley rats the 26-month feeding study of **Lankas (1981)**[74]

| Tumor | Sex | Doses (mg/kg/day) | | | | p-values |
|---|---|---|---|---|---|---|
| | Male | 0 | 3.05 | 10.30 | 31.49 | |
| | Female | 0 | 3.37 | 11.22 | 34.02 | |
| Testicular interstitial cell tumors | Male | 0/50 | 3/50 | 1/50 | 6/50** | $P_{Trend}$=0.009 $P_{Hist}$=0.006 |
| Interstitial cell hyperplasia | Male | 1/50 | 1/50 | 1/50 | 0/50 | $P_{Trend}$=0.830 |
| Thyroid C-cell Carcinomas | Female | 1/47 | 0/49 | 2/50 | 6/47 | $P_{Trend}$=0.003 $P_{Hist}$=<0.001 |
| Thyroid C-cell Adenomas and Carcinomas | Female | 6/47 | 3/49 | 8/50 | 9/47 | $P_{Trend}$=0.072 $P_{Hist}$=0.072 |
| Pancreas Islet Cell Tumors | Male | 0/50 | 5/50* | 2/50 | 3/50 | $P_{Trend}$=0.312 |
| lymphocytic hyperplasia, thymus and lymph nodes | Female | 27/50 | 35/50 | 38/50* | 35/50 | $P_{Trend}$=0.143 |
| Thyroid C-cell Adenomas and Carcinomas | Male | 1/47 | 2/49 | 4/49 | 4/49 | $P_{Trend}$=0.122 |
| Thyroid Follicular-cell Adenoma | Male | 5/47 | 1/49 | 2/49 | 2/49 | $P_{Trend}$=0.748 |
| Liver Neoplastic Nodule | Male | 3/50 | 5/50 | 1/50 | 3/10 | $P_{Trend}$=0.630 |
| Kidney Adenoma | Male | 1/50 | 5/50 | 0/50 | 0/50 | $P_{Trend}$=0.979 |

*- $p_{Fisher}$<0.05, **- $p_{Fisher}$<0.01

In conclusion, this study shows positive result for testes interstitial cell tumors and hepatocellular adenomas 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.

**Stout and Ruecker (1990)**[78] exposed groups of 50 male and 50 female Sprague-Dawley rats to glyphosate (98.7% purity) in feed (see Table 2 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 2). However, these rates include the 10 animals that were sacrificed at one year. Due to the short duration of exposure, the rats terminated at one year were likely not at risk of developing this tumor; it is very unusual to include these animals in the final tumor counts (EPA[61] also excluded these animals). In the pathology tables for this study, there were no tumors in any of the 10 animals at the interim sacrifice. Removing these 10 animals does not alter the p-values for trend or

Fisher's exact test. Historical control data for this tumor in this laboratory was reported as 23/432 or 5.3%[96] and a trend comparison against this control rate was not significant ($p_{hist}$=0.15). 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)**[74] 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 2) 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[6] in adenomas and carcinomas combined ($p_{Trend}$=0.052) regardless of whether interim sacrificed animals are included (Table 2). In males, the trend for adenomas was $p_{Trend}$=0.084 and for adenomas and carcinomas was $p_{Trend}$ =0.091. 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.

Several other tumors demonstrating significant findings in other studies of Sprague-Dawley rats are included in Table 2 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 adenomas in male rats with no increases in carcinomas could be due to chance. The findings of significant increases in thyroid c-cell tumors in males and females should be compared with other studies. This study will be included in the overall evaluation of causation.

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

**Table 2:** Tumors of interest in male and female Sprague-Dawley rats from the 24-month feeding study of Stout and Ruecker (1990)[78]

| Tumor | Sex | Doses (mg/kg/day) | | | | p-values |
|---|---|---|---|---|---|---|
| | Male | 0 | 89 | 362 | 940 | |
| | Female | 0 | 113 | 457 | 1183 | |
| Pancreas Islet Cell Tumors (with interim sacrifice) | Male | 1/58 | 8/57* | 5/60 | 7/59* | $P_{Trend}$=0.147 $P_{Hist}$=0.140 |
| Pancreas Islet Cell Tumors (without interim sacrifice) | Male | 1/48 | 8/47* | 5/50 | 7/49* | $P_{Trend}$=0.147 $P_{Hist}$=0.150 |
| Hepatocellular adenomas (without interim sacrifice) | Male | 3/50 | 2/50 | 3/50 | 8/50 | $P_{Trend}$=0.015 |
| Hepatocellular Adenomas and Carcinomas (without interim sacrifice) | Male | 6/50 | 4/50 | 4/50 | 10/50 | $P_{Trend}$=0.050 |
| Thyroid C-Cell Adenomas (with interim sacrifice) | Female | 2/60 | 2/60 | 6/60 | 6/60 | $P_{Trend}$=0.050 |
| Thyroid C-Cell Adenomas (without interim sacrifice) | Female | 2/50 | 2/50 | 6/50 | 6/50 | $P_{Trend}$=0.049 |
| Thyroid C-Cell Adenomas and Carcinomas (with interim sacrifice) | Female | 2/60 | 2/60 | 7/60 | 6/60 | $P_{Trend}$=0.053 |
| Thyroid C-Cell Adenomas and Carcinomas (without interim sacrifice) | Female | 2/50 | 2/50 | 7/50 | 6/50 | $P_{Trend}$=0.052 |
| Thyroid C-Cell Adenomas (with interim sacrifice) | Male | 2/60 | 4/60 | 8/60 | 7/60 | $P_{Trend}$=0.063 |
| Thyroid C-Cell Adenomas (without interim sacrifice) | Male | 0/50 | 4/50 | 8/50** | 5/50* | $P_{Trend}$=0.084 |
| Thyroid C-Cell Adenomas and Carcinomas (with interim sacrifice) | Male | 2/60 | 6/60 | 8/60* | 8/60* | $P_{Trend}$=0.068 |
| Thyroid C-Cell Adenomas and Carcinomas (without interim sacrifice) | Male | 0/50 | 6/50* | 8/50** | 6/50* | $P_{Trend}$=0.091 |
| Testis Interstitial Cell Tumors | Male | 2/50 | 0/50 | 3/50 | 2/50 | $P_{Trend}$=0.296 |
| Kidney Adenomas | Males | 0/50 | 2/50 | 0/50 | 0/50 | $P_{Trend}$=0.813 |
| Thyroid Follicular Adenoma/Carcinoma | Males | 2/50 | 1/48 | 3/48 | 3/50 | $P_{Trend}$=0.225 |

*- $p_{Fisher}$<0.05, **- $p_{Fisher}$<0.01

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**Atkinson et al. (1993)**[68] 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 3. 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.

**Table 3:** Tumors of interest in male and female Sprague-Dawley rats from the 24-month feeding study of Atkinson et al. (1993)[68]

| Tumor | Sex | Doses (mg/kg/day) | | | | | p-values |
|---|---|---|---|---|---|---|---|
| | Male | 0 | 11 | 112 | 320 | 1147 | |
| | Female | 0 | 12 | 109 | 347 | 1134 | |
| Thyroid Follicular Adenomas and Carcinomas | Male | 0/50 | 0/21 | 0/17 | 2/21 | 2/49 | $P_{Trend}$=0.099 |
| Thyroid Follicular Adenomas and Carcinomas (adding terminal sacrifice animals to denominator) | Male | 0/50 | 0/50 | 0/50 | 2/50 | 2/49 | $P_{Trend}$=0.034 |
| Thyroid C-cell Adenomas and Carcinomas | Female | 8/50 | 1/27 | 1/29 | 1/29 | 7/49 | $P_{Trend}$=0.197 |
| Thyroid C-cell Adenomas and Carcinomas | Male | 9/50 | 1/21 | 1/17 | 2/21 | 9/49 | $P_{Trend}$=0.183 |
| Testes Interstitial Cell Tumors | Male | 3/50 | 1/25 | 0/19 | 0/21 | 2/50 | $P_{Trend}$=0.580 |
| Kidney Adenomas | Males | 1/50 | 0/50 | 0/50 | 0/50 | 0/50 | $p_{Trend}$=1 |
| Hepatocellular Adenomas | Males | 2/50 | 1/50 | 1/50 | 2/50 | 3/50 | $P_{Trend}$=0.155 |
| Pancreas Islet-Cell Adenoma | Male | 0/50 | 0/50 | 0/50 | 0/50 | 1/50 | $P_{Trend}$=0.200 |

*- $p_{Fisher}$<0.05, **- $p_{Fisher}$<0.01

The authors reported no significant effects, as do **EPA**[61] and **EFSA**[89].  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 provided in **Greim et al. (2015)**[91], 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).

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.

**Brammer (2001)**[69] 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

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Table 4 . 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[97] (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**[61], but not **EFSA**[89], 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)**[91] 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.  These numbers have been included in Table 4, but it does not change the significance of the findings.  **Greim et al. (2015)**[91] 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.

**Table 4:** Tumors of interest in male and female Wistar rats from the 24-month feeding study of  Brammer (2001)[69]

| Tumor | Sex | Doses (mg/kg/day) | | | | p-values |
|---|---|---|---|---|---|---|
| | Male | 0 | 121 | 361 | 1214 | |
| | Female | 0 | 145 | 437 | 1498 | |
| Hepatocellular Adenoma | Male | 0/52 | 2/52 | 0/52 | 5/52* | $P_{Trend}$=0.008 |
| Hepatocellular Adenoma (from Greim et al., 2015[91]) | Male | 0/53 | 2/53 | 0/53 | 5/52* | $P_{Trend}$=0.008 $P_{Hist}$=0.006 |
| Mammary Gland Adenomas and Adenocarcinomas | Female | 3/51 | 2/51 | 0/51 | 2/51 | $P_{Trend}$=0.575 |
| Skin Keratocanthoma | Male | 1/51 | 0/51 | 1/51 | 1/51 | $P_{Trend}$=0.392 |

 *- $p_{Fisher}$<0.05, **-  $p_{Fisher}$<0.01

I obtained historical control data from 16 control  groups in Wistar rats from Charles River Laboratories for the years 2003 to 2011[98].  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

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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).  Assuming the underlying probability of having a tumor in controls is 4.27%, $p_{Hist}$=0.006 (Table 4). 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.

**Pavkov and Wyand (1987)**[75] 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[61]. 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)**[79] 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 5.

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

**EPA**[61] concluded there were no tumors increased due to glyphosate exposure in this study and **EFSA**[89] 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)**[91] provided data on hepatocellular adenomas and carcinomas in both sexes but none of these showed significant trends or pairwise tests (Table 5). However, there was another study with a strong significant trend in hepatocellular adenomas in Wistar rats[69] so these are also included in Table 5 for comparison.  No other tumors were mentioned by any other group and an examination of the grouped pathology tables provided by **Greim et al. (2015)** show an increase in mammary gland adenomas at the mid-dose ($p_{Fisher}$=0.017) but no significant trend. However, there was another study with a strong significant trend in mammary gland adenomas and adenocarcinomas combined in Wistar rats[80] so these are also included in Table 5 for comparison.  Like the **Atkinson et al. (1993)**[68] study, **Suresh (1996)** did not do full pathology on all of the animals in the interim exposure groups making

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interpretation of this study problematic.

This study 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 Suresh(1996)[79]

| Tumor | Sex | Doses (mg/kg/day) | | | | p-values |
|---|---|---|---|---|---|---|
| | Male | 0 | 6.3 | 59.4 | 595.2 | |
| | Female | 0 | 8.6 | 88.5 | 886 | |
| Mammary Gland Adenoma and Carcinoma | Female | 5/40 | 3/28 | 8/33 | 2/48 | $P_{Trend}$=0.970 |
| Hepatocellular Adenoma | Male | 24/50 | 22/50 | 10/50 | 21/50 | $P_{Trend}$=0.374 |
| Skin Keratocanthoma | Male | 0/50 | 0/50 | 0/50 | 0/50 | $P_{Trend}$=1 |

\*- $p_{Fisher}$<0.05, \*\*- $p_{Fisher}$<0.01

**Enemoto (1997)**[72] 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 6). 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 included in the analysis if tumors were seen in these groups.

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 6). Examining the pathology tables provided in **Greim et al. (2015)** reveals no additional tumors showing an increase in tumor incidence with dose.  A different study[74] in Sprague-Dawley rats demonstrated a strong significant trend in mammary gland adenomas, thyroid C-cell carcinomas, skin Keratocanthomas and testicular interstitial cell tumors so these are also included in Table 6 for comparison.

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

**Table 6:** Tumors of interest in male and female Sprague-Dawley rats from the 24-month feeding study of  Enemoto (1997)[72]

| Tumor | Sex | Doses (mg/kg/day) | | | | p-values |
|---|---|---|---|---|---|---|
| | Male | 0 | 104 | 354 | 1127 | |
| | Female | 0 | 115 | 393 | 1247 | |
| Mammary Gland Adenoma | Female | 23/50 | 27/50 | 24/50 | 30/50 | $P_{Trend}$=0.106 |
| Kidney Adenoma | Male | 0/50 | 0/50 | 0/50 | 4/50 | $P_{Trend}$=0.004 |
| Thyroid C-cell Adenomas/Carcinomas | Female | 4/60 | 7/60 | 8/60 | 4/60 | $P_{Trend}$=0.692 |
| Thyroid C-cell Adenomas/Carcinomas | Male | 8/70 | 10/70 | 6/70 | 7/70 | $P_{Trend}$=0.697 |
| Thyroid Follicular-cell Adenomas/Carcinomas | Male | 4/70 | 2/70 | 1/70 | 0/70 | $P_{Trend}$=0.990 |
| Testes Interstitial Cell Tumors | Male | 3/49 | 2/50 | 0/50 | 2/50 | $P_{Trend}$=0.594 |
| Hepatocellular Adenomas | Male | 1/60 | 0/60 | 2/60 | 1/60 | $P_{Trend}$=0.371 |
| Skin Keratocanthoma | Male | 3/50 | 3/50 | 0/50 | 6/50 | $P_{Trend}$=0.065 |
| Pancreas Islet-Cell Adenoma | Male | 4/50 | 1/50 | 2/50 | 1/50 | $P_{Trend}$=0.844 |

   *- $p_{Fisher}$<0.05, **-  $p_{Fisher}$<0.01

**Wood et al. (2009)[80]** 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 7.

No survival differences were seen in this study.

 **EFSA**[89] found no dose-related tumor increases while **EPA**[61] 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 7).  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. It seems likely that, in this case, mammary gland adenocarcinomas can arise without the presence of any adenomas. **Greim et al (2015)**[91] also noted an increase in skin keratoacanthoma in males ($p_{Trend}$=0.030).  Review of the pathology tables identified no other tumors with increased tumor rates as a function of dose. There was another study with a strong significant trend in hepatocellular adenomas in Wistar rats[69] so this tumor is also included in Table 7 for comparison.

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

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**Table 7:** Tumors of interest in male and female Wistar rats from the 24-month feeding study of Wood et al. (2009)[80]

| Tumor | Sex | Doses (mg/kg/day) | | | | p-values |
|---|---|---|---|---|---|---|
| | Male | 0 | 85.5 | 285.2 | 1077.4 | |
| | Female | 0 | 104.5 | 348.6 | 1381.9 | |
| Mammary Gland Adenomas | Female | 0/51 | 0/51 | 0/51 | 2/51 | $P_{Trend}$=0.062 |
| Mammary Gland Adenocarcinomas | Female | 2/51 | 3/51 | 1/51 | 6/51 | $P_{Trend}$=0.042 |
| Mammary Gland Adenomas and Adenocarcinomas | Female | 2/51 | 3/51 | 1/51 | 8/51* | $P_{Trend}$=0.007 |
| Skin Keratocanthoma | Male | 2/51 | 3/51 | 0/51 | 6/51 | $P_{Trend}$=0.030 |
| Hepatocellular Adenoma | Male | 0/51 | 2/51 | 1/51 | 1/51 | $P_{Trend}$=0.418 |

*- $p_{Fisher}$<0.05, **- $p_{Fisher}$<0.01

**Excel (1997)[73]** 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**[61], **EFSA**[89] and **Greim et al. (2015)**[91] 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)[71]** 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)**[91], 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)**[91] 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)[77]** exposed Sprague-Dawley rats to the glyphosate formulation Roundup in drinking water for two years as part of a broader experiment on

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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.

**Joint Analysis - Rats**

Table 8 summarizes the significance for all tumors of interest in rats.

**Brammer** (2001)[69] saw a significant increase in hepatocellular adenomas in male Wistar rats with increasing dose ($p_{Trend}$=0.008, Table 4).  The other two acceptable studies in Wistar rats (**Wood et al. (2009)**[80] and **Suresh (1996)**[79] did not see significant increases (Tables 5 and 7). On the basis of statistical significance, these studies are inconsistent. To reject these findings based upon only 1/3 being positive is the same as rejecting a coin as being fair if, in three flips of the coin, the result is one head and two 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.  **Suresh (1996)** 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, **Suresh (1996)** has a significantly different control response from the other two.  **Suresh (1996)** did not give a substrain for the Wistar rats used, but **Brammer (2001)** and **Wood et al. (2009)** 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 **Suresh (1996).** It is known that the same strain of rats from different laboratories can have markedly different control tumor responses.  Because they have similar control response, **Brammer (2001)** and **Wood et al. (2009)** can be pooled into a single study to ask the question "Does the significant trend for **Brammer (2001)** disappear when it is pooled with the negative study of **Wood et al. (2009)**?"  The analysis of the pooled studies yields $p_{Trend}$=0.013 supporting the conclusion that glyphosate causes hepatocellular adenomas in Wistar rats with similar background responses.

**Wood et al. (2009)**[80] saw a significant increase in mammary gland adenomas and adenocarcinomas ($p_{Trend}$=0.007, Table 7) in females that was not seen in the other two studies (Tables 4 and 6).  The background rates in these studies differ only slightly and a pooled analysis of all three studies yields $p_{TrendA}$=0.459, suggesting that combining the data eliminates the dose-response trend seen in **Wood et al. (2009)**.  However, if the Wistar rats used in **Suresh (1996)** differed in their response for hepatocellular adenomas, they may differ for this tumor as well.  Combining only **Wood et al. (2009)** with **Brammer (2001)** results in $p_{Trend}$=0.037.  Given the mixed results from the pooling for this tumor I conclude there is limited support for the notion that glyphosate can cause mammary gland adenomas and adenocarcinomas in Wistar rats.

**Wood et al. (2009)[80]** saw a significant increase in skin keratocanthomas ($p_{Trend}$=0.030, Table 7) in males that was not seen in the other two studies (Tables 4 and 6). The background rates in these studies differ only slightly and a pooled analysis of all three studies yields $p_{TrendA}$=0.010, suggesting that combining the data does not eliminate the dose-response trend seen in **Wood et al. (2009)**. Combining only **Wood et al. (2009)** with **Brammer (2001)** results in $p_{Trend}$=0.053. Given the results from the pooling for this tumor I conclude there is support for the notion that glyphosate can cause skin keratocanthomas in Wistar rats.

In Sprague-Dawley rats, there were four studies that were acceptable for inclusion in the evaluation of causality with one[74] yielding strong positive responses for thyroid C-cell carcinomas in females and testicular interstitial tumors and hepatocellular adenomas in males and another[72] yielding a strong result for kidney adenomas in males. **Lankas (1981)**[74] saw a significant increase in thyroid C-cell carcinomas in female rats exposed to glyphosate ($p_{Trend}$=0.003, Table 1) and a marginal increase in C-cell adenomas and carcinomas combined ($p_{Trend}$=0.072, $p_{hist}$=0.072, Table 1; one of the other three studies also saw marginal results for thyroid C-cell adenomas and carcinomas in females (Table 2). A pooled analysis using all four studies yields $p_{TrendA}$=0.390. This pooled analysis does not support the results seen in **Lankas (1981)**. However, the **Lankas (1981)** study 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. From these data, I conclude that the evidence is weak that glyphosate causes thyroid C-cell tumors in female Sprague-Dawley rats.

Thyroid C-cell adenomas and carcinomas combined, in males, show marginally significant dose-response trends in **Stout and Ruecker (1990**, Table 2) but not in the remaining three studies. Pooling all four studies yields a significant trend of $p_{TrendA}$=0.041. From these data, I conclude that there is evidence is that glyphosate causes thyroid C-cell tumors in male Sprague-Dawley rats.

Thyroid follicular-cell adenomas and carcinomas combined, in males, show a significant dose-response trend in **Atkinson et al. (1993**, Table 3) but not in the remaining three studies;. Pooling all four studies yields no significant trend with $p_{TrendA}$=0.618. From these data, I conclude that there is no evidence that glyphosate causes thyroid follicular-cell tumors in male Sprague-Dawley rats.

Hepatocellular adenomas, in males, show a significant dose-response trend in **Stout and Ruecker (1990**, Table 2) but not in the remaining three studies. Pooling all four studies yields a marginally significant trend with $p_{Trend}$=0.073. From these data, I conclude that there is limited evidence that glyphosate causes thyroid follicular-cell tumors in male Sprague-Dawley rats.

**Table 8:** Summary of significance tests for 5 tumors from 7 studies in Rats

| Study | Strain | Neoplasm | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Hepato-cellular Adenomas (males) | Mammary Gland Tumors (females) | Skin Kerato-canthoma (males) | Thyroid C-Cell Tumors (females) | Thyroid C-Cell Tumors (males) | Thyroid Follicular Cell Tumors (males) | Testis Inter-stitial Cell Tumors (male) | Kidney Adenomas (males) |
| Brammer (2001)[69] | Wistar | +++[1] | - | | | | | | |
| Wood (2009)[80] | | - | +++ | ++ | | | | | |
| Suresh (1996)[79] | | - | - | | | | | | |
| Pooled Wistar Rats | | ++[2] | ++[2] | +++ | | | | | |
| Lankas (1981)[74] | Sprague Dawley | -[3] | | | + | - | - | +++ | - |
| Enemoto (1997)[72] | | - | | | - | - | - | - | +++ |
| Atkinson et al. (1993)[68] | | - | | | - | - | ++ | - | - |
| Stout and Ruecker (1990) | | ++ | | | + | + | - | - | - |
| Pooled Sprague-Dawley Rats | | + | | | - | ++ | - | - | ++[4] |

[1]entries are $p_{Trend}/p_{Hist}$ with values: – $p>0.1$, + $0.1{\geq}p>0.05$, ++ $0.05{\geq}p>0.01$, +++ $p{\leq}0.01$; [2]pooling results from **Brammer (2001)** and **Wood (2009)** only; [3]liver neoplastic nodules; [4]excluding **Lankas (1981)**

Another significant trend seen in Sprague-Dawley rats is the finding of testes interstitial cell tumors from **Lankas (1981)**[74] ($p_{Trend}$=0.009, Table 1); the other three studies were negative for this tumor (Tables 2, 3 and 6). Combining the other three studies with that of **Lankas (1981)** for testes interstitial tumors results in a p-value for trend that is clearly non-significant ($p_{TrendA}$=0.608). However, as noted above, the **Lankas (1981)** study 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 to **Stout and Ruecker (1990)**, **Atkinson et al.(1993)** and **Enemoto (1997)**.

The final tumor in Sprague-Dawley rats showing a strong significant trend is kidney

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adenomas in males from the study by **Enemoto (1997)**[72] ($P_{Trend}$=0.004, Table 6).  The kidney tumor data is not significant for the studies by **Lankas (1981)**[74] (Table 1), **Atkinson et al. (1993)**[99] (Table 3) and **Stout and Ruecker (1990)**[78] (Table 2).  Pooling the **Enemoto (1997)** study with that of **Lankas (1981)**[74], **Stout and Ruecker (1990)** and **Atkinson et al. (1993)** yields $p_{TrendA}$=0.201.  Removing the 26-month study by **Lankas (1981)**[74] yields a p-value for the three combined 24-month studies of $p_{Trend}$=0.031; thus, the association between glyphosate and kidney adenomas in male Sprague-Dawley rats is supported by these data, even with the difficulty associated with interpreting the results in the low- and mid-doses in the **Atkinson et al. (1993)** study.  There is evidence to support an increase in kidney tumors in male Sprague-Dawley rats exposed to glyphosate.

In summary, there is evidence that glyphosate causes hepatocellular adenomas and skin keratocanthomas in male Wistar rats, mammary gland adenomas and adenocarcinomas in female Wistar rats and kidney adenomas and thyroid C-cell adenomas and carcinomas in male Sprague-Dawley rats.  There is limited evidence glyphosate causes hepatocellular adenomas in male Sprague-Dawley rats.


**Mouse Studies**

**Reyna and Gordon (1974)**[86] 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.  However, 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)**[83] 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 9).

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


 **EPA**[100] found a significant increase in kidney tubular cell adenomas in male mice based upon the original pathology done from the study and this analysis is shown in Table 9 ($p_{Trend}$=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.  No historical controls were available from the laboratory that conducted **Knezevich and Hogan, (1983)** so IARC, EPA and EFSA all used historical control databases from published studies in the

literature[101-103].  These studies have virtually identical rates for the important tumors seen in CD-1 mice; I will use the study by **Giknis and Clifford (2000)**[102] since it best covers the range of studies we have for CD-1 mice.  For studies of approximately two years, the mean historical tumor response in controls is 0.27%.  Applying this control response rate to the kidney adenomas yields $p_{Hist}$=0.005, strengthening the significance of the evaluation against the concurrent control.  EPA originally used a similar analysis and reached the same conclusions.  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 9).  With these changes, the adenomas no longer have a significant trend ($P_{Trend}$=0.442, $P_{Hist}$=0.121) but carcinomas have a marginally significant trend against concurrent controls and a clearly significant trend using historical controls ($p_{Trend}$=0.063, $p_{Hist}$=0.002, historical control rate of 0.15%).  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[104].  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, $p_{Hist}$=0.011, historical control rate of 0.44%).  However, there was considerable disagreement on whether the one adenoma in the control group was correctly diagnosed[105].  Removing this one adenoma from the control group results in $p_{Trend}$=0.019 and $p_{Hist}$=0.005.

Other CD-1 mouse studies have seen increases in malignant lymphomas, hemangiosarcomas and lung adenocarcinomas (males) and hemangiomas (females).  Evaluations of those tumors for this study yields results that are not significant; for malignant lymphoma, $p_{Trend}$=0.754, $p_{Hist}$=0.767, with the historical control rate equal 6.2%, for hemangiosarcomas $p_{Trend}$=0.503, $p_{Hist}$=0.591, with the historical control rate equal to 2.5%, for lung adenocarcinomas $p_{Trend}$=0.918, $p_{Hist}$=0.899, with the historical control rate equal to 9.2% and for hemangiomas $p_{Trend}$=0.631.  No other tumors were found in this study.

The **EPA**[61] 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 there are two very good historical control databases for CD-1 mice[101, 102].  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, Table 9). EPA then states, without reference, that *"chronic interstitial nephritis is not considered to be a precursor lesion for tubular neoplasms"*. I could find no published research 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

**Table 9:** Tumors of interest in male and female CD-1 mice from the 24-month feeding study of **Knezevich and Hogan (1983)**[83]

| Tumor | Sex | Doses (mg/kg/day) | | | | p-values |
|---|---|---|---|---|---|---|
| | Male | 0 | 157 | 814 | 4841 | |
| | Female | 0 | 190 | 955 | 5874 | |
| Kidney Adenoma[1] (original pathology) | Male | 0/49 | 0/49 | 1/50 | 3/50 | $P_{Trend}$=0.019 $P_{Hist}$=0.005 |
| Kidney Adenoma (EPA pathology) | Male | 1/49 | 0/49 | 0/50 | 1/50 | $P_{Trend}$=0.442 $P_{Hist}$=0.121 |
| Kidney Carcinoma[2] (EPA pathology)u | Male | 0/49 | 0/49 | 1/50 | 2/50 | $P_{Trend}$=0.063 $P_{Hist}$=0.002 |
| Kidney Adenoma and Carcinoma Combined[3] (EPA pathology) | Male | 1/49 | 0/49 | 1/50 | 3/50 | $P_{Trend}$=0.065 $P_{Hist}$=0.011 |
| Malignant Lymphoma[4] | Male | 2/49 | 5/49 | 4/50 | 2/50 | $P_{Trend}$=0.754 $P_{Hist}$=0.767 |
| Hemangiosarcoma[5] | Male | 0/50 | 0/49 | 1/50 | 0/50 | $P_{Trend}$=0.503 $P_{Hist}$=0.591 |
| Bilateral Chronic Interstitial Nephritis | Male | 5/49 | 1/49 | 7/50 | 11/50 | $P_{Trend}$=0.006 |
| Hemangiooma[6] | Female | 0/49 | 1/49 | 1/50 | 0/50 | $P_{Trend}$=0.631 |
| Lung Adenocarcinoma[7] | Male | 4/48 | 3/50 | 2/50 | 1/50 | $P_{Trend}$=0.918 $P_{Hist}$=0.899 |

*- $p_{Fisher}$<0.05, **- $p_{Fisher}$<0.01, [1]historical rate=0.27%, [2]historical rate=0.15%, [3]historical rate=0.44%, [4]historical rate=6.2%, [5]historical rate=2.5%, [6]No Historical Controls, [7]Historical rate=9.2%

to play an important role in kidney cancer[106] and many other cancers so this argument also fails to support rejection of these findings.

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

**Atkinson, et al., (1993)**[81] 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 10).

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

**Table 10:** Tumors of interest in male and female CD-1 mice from the 24-month feeding study of  **Atkinson et al. (1993)**[81]

| Tumor | Sex | Doses (mg/kg/day) | | | | p-values |
|---|---|---|---|---|---|---|
| | Male | 0 | 98 | 297 | 988 | |
| | Female | 0 | 102 | 298 | 1000 | |
| Kidney Adenoma and Carcinoma Combined[1] | Male | 2/50 | 2/50 | 0/50 | 0/50 | $P_{Trend}$=0.981 $P_{Hist}$=1 |
| Malignant Lymphoma[2] | Male | 4/50 | 2/50 | 1/50 | 6/50 | $P_{Trend}$=0.087 $P_{Hist}$=0.085 |
| Hemangiosarcoma[3] | Male | 0/50 | 0/50 | 0/50 | 4/50 | $P_{Trend}$=0.004 $P_{Hist}$=0.001 |
| Hemangioma[4] | Female | 0/50 | 0/50 | 0/50 | 0/50 | $P_{Trend}$=1 |
| Lung Adenocarcinoma[5] | Male | 10/50 | 7/50 | 8/50 | 9/50 | $P_{Trend}$=0.456 $P_{Hist}$=0.449 |

\*- $p_{Fisher}$<0.05, \*\*- $p_{Fisher}$<0.01, [1]historical rate=0.44%, [2]historical rate=6.2%, [3]historical rate=2.5%, [4]No historical control rate, [5]Historical rate=9.2%

 Hemangiosarcomas were the only tumors showing a significant trend in this study ($P_{Trend}$=0.004, $P_{Hist}$=0.001, Table 10).  Also shown in Table 10 are the results for malignant lymphomas, kidney tumors and lung adenocarcinomas (males) and hemangioma (females); there is a marginal trend for malignant lymphomas ($P_{Trend}$=0.087, $P_{Hist}$=0.085) and no trend for kidney tumors.

The **EPA**[61] concluded the findings 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**[54]. I have already commented on how EPA's guidelines treat trend tests and Fisher's Exact test results, 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 is addressed directly by the evaluation against the historical control rates; if these rates were high enough to exclude this finding, $P_{Hist}$ would have be above 0.05 instead of 0.001.  The mean

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historical control incidence of hemangiosarcomas in controls from two-year cancer bioassays in CD-1 mice is 2.5% and the response seen in the high-dose group is 8.9%. The **SAP**[54] stated very clearly that the practice, being used by the EPA, of negating a positive finding because of historical control data was not acceptable[54]. (page 63). The EPA Cancer Guidelines[33] 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.*"

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

**Wood et al., (2009)**[88] 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 11).

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

No increase in kidney tumors or hemangiosarcomas (males) or hemangiomas (females) were seen in this study.  There was a monotonic increase in lung adenocarcinomas ($p_{Trend}$=0.028, $p_{Hist}$=0.031) in males and a monotonic increase in malignant lymphomas ($p_{Trend}$=0.007, $p_{Hist}$=0.007) in males.  The historical control incidence for this study is different from the earlier studies because this study is only for 80 weeks instead of 104 weeks (two years); the historical control rate for malignant lymphomas in CD-1 mice after 80 weeks is 2.6% instead of 6.2%, the historical control rate at two years[102].

For lung adenocarcinomas, the **EPA**[61] 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[107].  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**[61] uses historical control data[103, 108] 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)**[108] saw 21 animals out of 1453 examined prior to 80 weeks with lung adenocarcinomas (1.4%).  **Giknis and Clifford (2005)**[103] 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 (21 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%. **Giknis and Clifford (2000)**[102] (the reference I have been citing) 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**[33, 54] or its **SAP**[54].

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

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 11:** Tumors of interest in male and female CD-1 mice from the 18-month feeding study of  **Wood et al. (2009)**[88]

| Tumor | Sex | Doses (mg/kg/day) | | | | p-values |
|---|---|---|---|---|---|---|
| | Male | 0 | 71.4 | 234.2 | 810 | |
| | Female | 0 | 97.9 | 299.5 | 1081.2 | |
| Kidney Adenoma[1] | Male | 0/51 | 0/51 | 0/51 | 0/51 | $P_{Trend}$=1 |
| Malignant Lymphoma[2] | Male | 0/51 | 1/51 | 2/51 | 5/51* | $P_{Trend}$=0.007 $P_{Hist}$=0.007 |
| Hemangiosarcoma | Male | 0/51 | 0/51 | 0/51 | 0/51 | $P_{Trend}$=1 |
| Lung Adenocarcinoma[3] | Male | 5/51 | 5/51 | 7/51 | 11/51 | $p_{Trend}$=0.028 $P_{Hist}$=0.031 |
| Hemangioma[4] | Female | 0/51 | 2/51 | 0/51 | 1/51 | $p_{Trend}$=0.438 |
| Animals with Malignant Neoplasms | Male | 14/51 | 20/51 | 17/51 | 20/51 | $P_{Trend}$=0.203 |
| Animals with Malignant Neoplasms | Female | 23/51 | 15/51 | 17/51 | 18/51 | $P_{Trend}$=0.628 |
| Animals with multiple malignant tumors | Male | 1/51 | 2/51 | 3/51 | 5/51 | $P_{Trend}$=0.046 |

*- $p_{Fisher}$<0.05, **-  $p_{Fisher}$<0.01, [1]historical rate=0.44%, [2]historical rate=2.6%, [3]Historical rate=2.5%, [4]No Historical Control Rate

**Sugimoto (1997)**[87] 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 12).

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

Kidney adenomas ($p_{Trend}$=0.062, $p_{Hist}$=0.005), malignant lymphomas ($p_{Trend}$=0.016,

$p_{Hist}$=0.017) and hemangiosarcomas ($p_{Trend}$=0.062, $p_{Hist}$=0.004) in male mice and hemangiomas ($p_{Trend}$=0.002) in female mice all showed increased tumor incidence with increasing dose.   The evaluation of lung adenocarcinomas in males showed no significant dose-related trend ($p_{Trend}$=0.148, $p_{Hist}$=0.140).  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)** so the development of an estimate of the historical control response is difficult (if the historical control rate is 0, then any observed response other than 0 has a p-value of 0). The fact that this tumor was never seen in the historical controls should strongly support any positive finding as being significant.  However, to still allow for a test using historical control data, I used the historical control estimate of the mean response that would result in a 5% chance of seeing no tumors in 1149 animals.  This estimated historical control response value was 0.0026.  This value was used in the analysis for hemangiosarcomas in male CD-1 mice exposed for 18 months ($p_{Hist}$ <0.001).

**Table 12:** Tumors of interest in male and female CD-1 mice from the 18-month feeding study of  **Sugimoto (1997)**[87]

| Tumor | Sex | Doses (mg/kg/day) | | | | p-values |
|---|---|---|---|---|---|---|
| | Male | 0 | 165 | 838.1 | 4348 | |
| | Female | 0 | 153.2 | 786.8 | 4116 | |
| Kidney Adenoma[1] | Male | 0/50 | 0/50 | 0/50 | 2/50 | $P_{Trend}$=0.062 $P_{Hist}$=0.005 |
| Malignant Lymphoma[2] | Male | 2/50 | 2/50 | 0/50 | 6/50 | $P_{Trend}$=0.016 $P_{Hist}$=0.017 |
| Hemangiosarcoma[3] | Male | 0/50 | 0/50 | 0/50 | 2/50 | $P_{Trend}$=0.062 $P_{Hist}$=0.004 |
| Hemangioma[4] | Female | 0/50 | 0/50 | 2/50 | 5/50* | $P_{Trend}$=0.002 |
| Lung Adenocarcinoma[5] | Male | 1/50 | 1/50 | 6/50 | 4/50 | $P_{Trend}$=0.148 $P_{Hist}$=0.140 |
| Number of animals with Malignant Neoplasms | Male | 5/50 | 5/50 | 11/50 | 16/50** | $P_{Trend}$=0.001 |
| Number of animals with Malignant Neoplasms | Female | 9/50 | 13/50 | 16/50 | 13/50 | $P_{Trend}$=0.362 |

*- $p_{Fisher}$<0.05, **- $p_{Fisher}$<0.01, [1]historical rate=0.44%, [2]historical rate=2.6%, [3]historical rate=0/1424 (0.26% - 95% confidence limit), [4]No Historical Control Rate, [5]Historical rate=2.5%

**EPA**[61] 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**[109]. 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.

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For the hemangiosarcomas in males, none of the 26 historical control groups examined by **Giknis and Clifford (2000)** had hemangiosarcomas, making this a very rare tumor in males prior to 80 weeks on study.  The malignant lymphomas in males are statistically significant against both the concurrent controls and the historical controls.  Finally, there is clearly an overall increase of malignancies in the males.

In summary, this study shows a positive result for kidney adenomas, malignant lymphomas and hemangiosarcomas in male CD-1 mice, hemangiomas 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.


**Kumar (2001)**[84] 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 13).

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 ($p_{Trend}$=0.062) and malignant lymphomas ($p_{Trend}$=0.064, $p_{Hist}$ =0.070) in male mice demonstrated marginal statistical significance and hemangiosarcomas ($p_{Trend}$=0.500) in male mice demonstrated no statistical significance. In this study, not all animals in the low- and mid- dose groups were evaluated for kidney tumors, so a second analysis was done based on only the animals examined in these two groups ($p_{Trend}$=0.088). No historical control data was available for hemangiosarcomas and kidney adenomas in Swiss Albino mice.  For the malignant lymphomas, EFSA provided a historical control data set showing a mean response of 46/250=0.184 (18.4%) with a range of 6% to 30%.  Using this historical control data, the trend is only marginally significant ($p_{Hist}$=0.070). I have some concern that 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.  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.

In a recent memo, Martens (2017) [110] asserts that the incidence counts for malignant lymphomas and kidney adenomas appearing in Greim et al. (2015)[91] and EFSA (2013)[89] are incorrect and provides different rates (shown in Table 13). The p-values for both of these tumors are reduced using the incidence counts from the Martens memo. However, it should be noted that if the counts for malignant lymphomas in the Martens (2017) memo are correct, then all three exposure groups have responses outside of the range of the historical controls.  It is unclear from Greim et al. (2015), EFSA or Martens (2017) which tumor incidence counts are correct.

There was a significant increase in hemangiomas (any tissue) in female mice ($p_{Trend}$=0.004).

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

**Table 13:** Tumors of interest in male and female Swiss Albino mice from the 18-month feeding study of **Kumar (2001)**[84]

| Tumor | Sex | Doses (mg/kg/day) | | | | p-values |
|---|---|---|---|---|---|---|
| | Male | 0 | 14.5 | 149.7 | 1453 | |
| | Female | 0 | 15 | 151.2 | 1466.8 | |
| Kidney Adenoma (only tissues examined microscopically) | Male | 0/50 | 0/26 | 1/22 | 2/50 | $P_{Trend}$=0.088 |
| Kidney Adenoma (as reported by Greim et al.) | Male | 0/50 | 0/50 | 1/50 | 2/50 | $P_{Trend}$=0.062 |
| Kidney Adenoma (as reported by Martens) | Male | 0/50 | 0/50 | 0/50 | 1/50 | $P_{Trend}$=0.250 |
| Malignant Lymphoma[1] (as reported by Greim et al.) | Male | 10/50 | 15/50 | 16/50 | 19/50 | $P_{Trend}$=0.064 $P_{Hist}$=0.070 |
| Malignant Lymphoma[1] (as reported by Martens) | Male | 10/50 | 16/50 | 18/50 | 19/50* | $P_{Trend}$=0.141 $P_{Hist}$=0.150 |
| Hemangiosarcoma | Male | 0/50 | 0/50 | 2/50 | 0/50 | $P_{Trend}$=0.500 |
| Hemangioma (any tissue) | Female | 1/50 | 0/50 | 0/50 | 5/50 | $P_{Trend}$=0.004 |

\*- $p_{Fisher}$<0.05, \*\*- $p_{Fisher}$<0.01, [1]Historical control rate=0.184 (46/250 mice)

evaluation of causation.

**Pavkov and Turner (1987)**[85] 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**[61] 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)**[91] 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)**[82] 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**[61] 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[54] 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.

**Joint Analysis - Mouse**

In their evaluation of the mouse studies, **EPA**[61] and **EFSA**[89] 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**[30], **EFSA**[90] extracted the original data and did trend tests on kidney tumors, malignant lymphomas and hemangiosarcomas in male mice in five of the mouse studies, the same five studies I consider acceptable for a causation analysis. 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.  I will now address both points.

In CD-1 mice, there are four 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 14 summarizes the positive and negative findings for all five 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.  For hemangiosarcomas in males, there are clear positive findings in the studies by **Sugimoto (1997)** and **Atkinson et al. (1993)** and non-significant responses in **Wood et al. (2009**) and **Knezevich and Hogan (1983)**.  In females, hemangiosarcomas are only present in the study by **Sugimoto (1997)**. Malignant lymphomas in males are clearly positive in two studies[87, 88] and marginally positive in a third[81] but negative in the fourth[83].  Both of the strong positive studies exposed animals for 18 months.  Kidney tumors in males are positive in two studies[83, 87] and negative in the remaining two[81, 88].  Lung adenocarcinomas in males are only positive in the study by **Wood et al. (2009)**.  **Sugimoto (1997)** had four clearly positive associations between tumors and glyphosate while the others had two or less.

**Table 14:** Summary of significance tests for 5 tumors from 4 studies in CD-1 Mice

| Study | Months on Study | Neoplasm | | | | |
|---|---|---|---|---|---|---|
| | | Hemangio-sarcoma (male) | Hemangioma (female) | Malignant Lymphoma (male) | Kidney Tumor (male) | Lung Adeno-carcinoma (male) |
| Sugimoto 1997[87] | 18 | +/+++[1] | +++ | ++/++ | +/+++ | -/- |
| Wood 2009[88] | 18 | -/- | - | +++/+++ | -/- | ++/++ |
| Sugimoto & Wood Pooled | | ++/+++ | +++ | +++/+++ | ++/+++ | -/- |
| Atkinson 1993[81] | 24 | +++/+++ | - | +/+ | -/- | -/- |
| Knezevich 1983[83] | 24 | -/- | - | -/- | +/++ | -/- |
| Atkinson & Knezevich Pooled | | -/- | - | -/- | +/+ | -/- |
| All CD-1 Studies Pooled | | ++/++ | ++/++ | +/+ | +++/+++ | -/- |

[1]entries are $p_{Trend}/p_{Hist}$ with values: – $p>0.1$, + $0.1≥p>0.05$, ++ $0.05≥p>0.01$, +++ $p≤0.01$

As seen for the rat studies, this simple evaluation of the positive versus negative findings fails to resolve the issue of which findings are driving the overall responses in these data.  To do this, I will again pool the studies.  Table 14 summarizes the pooled analyses.

For kidney tumors in males, pooling the two 18-month studies yields significant increases in incidence ($p_{Trend}$=0.015, $p_{Hist}$=0.003) and pooling of the two year studies shows marginal significance ($p_{Trend}$=0.081, $p_{Hist}$=0.054). Pooling all four studies results in ($p_{Trend}$=0.005, $p_{Hist}$=0.007), thus the positive trend remains. **Knezevich and Hogan (1983)** saw a 4% response for kidney carcinomas in their highest exposure group.  The largest response seen for kidney carcinomas in controls in 48 studies by **Giknis and Clifford (2000**) and in 52 studies by **Giknis and Clifford (2005)** was 2% and in the control groups from 11 two-year cancer studies, **Chandra and Frith (1992)**[101] saw only one animal out of 725 with a kidney carcinoma.   In 46 control datasets, **Giknis and Clifford (2000)** saw 39 control groups with no adenomas, five with one adenoma and two with two adenomas; both 24-month studies saw two adenomas in the highest exposure group, a very rare finding.  To better illustrate, there are 16 groups of animals in the four studies.  For any one group, there is a 2/44 or 4.3% chance of getting a response 4% or larger.  The chances of randomly getting 3 or more such responses in 16 groups is 2.9% and the chances of two of these being in any two of the four highest exposure groups is 0.01.  In summary, the strong finding in two of the four studies, the positive finding when all four studies are pooled and the very low probability that this is due to chance when compared to historical controls support the conclusion that glyphosate causes kidney tumors in male mice.

For malignant lymphomas in males, pooling the two 18-month studies, **Sugimoto (1997)** and **Wood et al. (2009)**, results in a significant trend ($p_{Trend}$=0.005, $p_{Hist}$=0.006).  Pooling the two 24-month studies, **Knezevich and Hogan (1983)** and **Atkinson et al. (1993)**, yields ($p_{Trend}$=0.653, $p_{Hist}$=0.649).  The main differences between these two findings is in the control response; the pooled control response at 24 months is 6/99 (6%) versus 2/101 at 18 months (2%). This is expected since, in the absence of any exposure, tumor rates increase as a function of age[5].  **Giknis and Clifford (2000)** show a control response at 18 months of 4% and a control response at 24 months of 6% (matching the value for the pooled studies). Pooling all four studies results in ($p_{TrendA}$=0.073, $p_{Hist}$=0.080). However, the responses seen for malignant lymphomas in controls by **Giknis and Clifford (2000)** show only one historical control group in twenty-six 18-month groups with 10% or higher response.  The responses at the high doses (10% and 12%) in the two 18-month studies are very unlikely to have arisen by chance.  There are eight groups of animals in the two studies.  For any one group, there is a 1/26 or 3.8% chance of getting a response of at least 10% based on the 26 control groups from **Giknis and Clifford (2000)**.  The chances of getting two or more such responses in eight groups is 0.035 and the chances of these being in three of the four highest exposure groups is 0.004. For the 24-month studies, the higher background rate makes it difficult to identify a small change in incidence, thus the findings in the 24-month studies and the 18-month studies are not inconsistent. In summary, the very strong findings in the 18-month studies, the very strong positive findings when the two 18-month studies are pooled,  the low probability that the responses seen in the 18-month studies are due to chance, and the

marginal increase in malignant lymphomas in the 18-month study in Swiss Albino mice[84] support the conclusion that glyphosate causes malignant lymphoma in male mice.

For hemangiosarcomas in males, pooling the two 18-month studies results in a significant trend ($p_{Trend}$=0.015, $p_{Hist}$=0.002).  Pooling the two 24-month studies yields ($p_{Trend}$=0.490, $p_{Hist}$=0.429).  The main difference between these two findings is the 0/50 response in animals exposed at 4841 mg/kg/day in the study by **Knezevich and Hogan (1983)**. Removing this one exposure group in the pooled 24-month analysis yields ($p_{Trend}$<0.001, $p_{Hist}$<0.001).  Pooling all four studies results in ($p_{Trend}$=0.045, $p_{Hist}$=0.043). No hemangiomas were seen in controls groups from twenty-six 18-month studies by **Giknis and Clifford (2000)** so the two hemangiosarcomas seen in the high dose group in the study by **Sugimoto (1997)** are biologically very significant.  For the 24-month historical controls, only two out of 20 control groups had a response greater than 8%. In summary, the very strong findings in the 18-month studies, the positive finding when all four studies are pooled and the low probability that the responses seen in the 18-month studies are due to chance support the conclusion that glyphosate causes hemangiosarcomas in male CD-1 mice.

For hemangiomas in females, pooling the two 18-month studies results in a significant trend ($p_{Trend}$=0.001).  Pooling the two-year studies results in $p_{Trend}$=0.424. Pooling all four studies results in $p_{Trend}$=0.018. In summary, the very strong findings in one 18-month study, the positive finding when all four studies are pooled and the low probability that the responses seen in the **Sugimoto (1997)** study are due to chance, support the conclusion that glyphosate causes hemangiomas in female CD-1 mice.

For lung adenocarcinomas in male CD-1 mice, pooling the two 18-month studies results shows no significant trend ($p_{Trend}$=0.417, $p_{Hist}$0.126).  Pooling the two 24 month studies yields ($p_{TrendA}$=0.985, $p_{Hist}$=0.993).  Pooling all four studies results in ($p_{TrendA}$=0.937, $p_{Hist}$=0.744). In summary, the moderate findings in one 24 month study, and the negative finding when any studies are pooled suggest that the linkage between glyphosate and lung adenocarcinomas in male CD-1 mice is due to chance.

The one study in Swiss Albino mice[84] was effectively negative for all endpoints except malignant lymphomas and kidney adenomas where marginally significant tumor responses were seen.  Considering the findings for kidney adenomas in CD-1 mice, glyphosate may also cause kidney adenomas in male Swiss Albino mice from the study of **Kumar (2001)**.

To summarize the findings in mice, glyphosate causes hemangiosarcomas, kidney tumors and malignant lymphomas in male CD-1 mice and hemangiomas in female CD-1 mice after 18 months of exposure, kidney tumors in male CD-1 mice after 24 months exposure and possibly kidney adenomas in male Swiss albino mice.  When 18-month and 24-month studies are pooled, there is a significant increase in hemangiosarcomas in male mice, hemangiomas in female mice and kidney tumors in male mice.

**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**[67] 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**[59] 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, there are no indications in any other studies of an exceedance in dose that would support ignoring the findings from any exposure group.

**EPA**[33] 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[33]

> "*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.

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Both of these guidelines make good scientific sense.  In the 12 acceptable rodent carcinogenicity studies included in this evaluation, no 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**[54].

Twenty chronic rodent carcinogenicity studies have been done using glyphosate as the test compound.  Eight of these studies are unacceptable for use in an evaluation of causality leaving seven studies in rats and five studies in mice.  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 15:** Observed versus expected tumor sites with significant trends in the 12 acceptable rodent carcinogenicity studies using glyphosate.

| Species | Strain | Sex | Total Sites[1] | Exp. <0.05 | Obs. <0.05 | Tumors[2] p<0.05 | Exp. <0.01 | Obs. <0.01 | Tumors p<0.01 |
|---|---|---|---|---|---|---|---|---|---|
| Rat (7 studies) | Sprague-Dawley (4 studies) | M | 86 | 4.3 | 4 | TICT, TFAC, KA, HA | 0.9 | 2 | TICT, KA |
| | | F | 102 | 5.1 | 1 | TCCC | 1.0 | 1 | TCCC |
| | Wistar (3 studies) | M | 64.5 | 3.2 | 2 | HA, SK | 0.6 | 1 | HA |
| | | F | 76.5 | 3.8 | 2 | MC, MAC | 0.8 | 1 | MAC |
| Mouse (5 studies) | CD-1 (4 studies) | M | 42 | 2.1 | 8 | KA, KC, KAC, HS(2)[3], ML(2), LAC | 0.4 | 5 | KA,KC, HS(2), ML |
| | | F | 60 | 3 | 1 | H | 0.6 | 1 | H |
| | Albino (1 study) | M | 10.5 | 0.5 | 0 | | 0.1 | 0 | |
| | | F | 15 | 0.8 | 1 | H | 0.2 | 1 | H |
| Rats (7 studies) | All (7 studies) | M | 150.5 | 7.5 | 6 | TICT, KA, HA(2), TFAC, SK | 1.5 | 3 | TICT, KA, HA |
| | | F | 178.5 | 8.9 | 3 | TCCC, MC, MAC | 1.8 | 2 | TCCC, MAC |
| | | Both | 329 | 16.5 | 9 | TICT, KA, HA(2), TFAC, SK, TCCC, MC, MAC | 3.3 | 5 | TICT, KA, HA, TCCC, MAC |
| Mice (5 studies) | All (5 studies) | M | 52.5 | 2.6 | 8 | KA, KC, KAC, HS(2), ML(2), LAC | 0.5 | 5 | KA,KC, HS(2), ML |
| | | F | 75 | 3.8 | 2 | H(2) | 0.7 | 2 | H(2) |
| | | Both | 127.5 | 6.4 | 10 | KA, KC, KAC, HS(2)[3], H(2), ML(2), LAC | 1.3 | 7 | KA,KC, HS(2), H(2), ML |
| All (12 studies) | All (12 studies) | M | 203 | 10.1 | 14 | TICT, KA(2), HA(2), TFAC, SK, KC, KAC, HS(2), ML(2), LAC | 2.0 | 8 | TICT, HA, KA(2),KC, HS(2), ML |
| | | F | 253.5 | 12.7 | 5 | TCCC, MC, MAC, H(2) | 2.5 | 4 | TCCC, MAC, H(2) |
| | | Both | 456.5 | 22.8 | 19 | TICT, KA(2), HA(2), TFAC, SK, KC, KAC, HS(2), H, ML(2), LAC, TCCC, MC, MAC | 4.6 | 12 | TICT, HA, KA(2), KC, HS(2), H(2), ML, TCCC, MAC |

[1]Number of sites examined is based upon suggestions by Dr. J. Haseman in his written testimony to the EPA; male mice – 10.5 sites; female mice – 15 sites; male rats – 21.5 sites; female rats – 25.5 sites

[2]Tumor abbreviations are: KA – kidney adenoma; KC – kidney carcinoma; KAC – kidney adenoma or carcinoma; HS – hemangiosarcoma; H – hemangioma; HA – hepatocellular adenoma; LAC – lung adenoma or adenocarcinoma; ML – malignant lymphoma; MC – mammary gland carcinoma; MAC – mammary gland adenoma or carcinoma; TCCC – thyroid C-cell carcinoma; TFAC

– thyroid follicular cell adenoma or carcinoma; TICT – testes interstitial cell tumor; SK – skin keratocanthoma
[3](x): x studies with this result

The EPA asked the SAP to comment on its evaluation of glyphosate[61] at a meeting in Washington, DC in December 2016[54].  Many comments were received from outside experts at this meeting; one such set of comments came from Dr. J. K. **Haseman (2016)**[111]. **Haseman (2016)** directly addressed the false-positive error rate and concluded that the results seen in these studies were due to chance.  He did this by deciding how many evaluations were likely for each study (broken into sex-by-species groups) and then aggregating the findings.  He concluded that the effective number of analyses were 10.5 in male mice, 15 for female mice, 21.5 for male rats, and 25.5 for female rats.  **Haseman (2016)** made two assumptions in his analysis that are not valid.  The first was that all of the possible trend tests had been done on all of the sites he considered reasonable for such an evaluation.  He identified eight positive findings. However, EPA had not evaluated all of the sites nor had they considered doing a formal analysis using historical control data.  EPA identified eight sex/species groups that had at most one positive tumor finding using the trend test with $p_{Trend} \leq 0.05$.  In Tables 1-14 above, I have identified 19 tumors with $p_{Trend} \leq 0.05$ or $p_{Hist} \leq 0.05$ and 12 with $p_{Trend} \leq 0.01$ or $p_{Hist} \leq 0.01$ (Table 15).  Secondly, Dr. Haseman assumed one could aggregate all the studies into one large analysis of Type-1 error. However, inference in these studies is always made by sex/species/strain (e.g. glyphosate causes hemangiosarcomas in male CD-1 mice; not glyphosate causes cancer in rodents), and the  analysis should have been done by grouping each separately.  Table 15 shows these analyses as well as the aggregated analysis for all of the acceptable studies.

With the exception of male Sprague-Dawley rats, the observed number of tumors are at or near the expected number for the different sex/strain groups in rats (Table 15).  For male Sprague-Dawley rats, 0.8 cases with $p_{Trend} \leq 0.01$ or $p_{Hist} \leq 0.01$ are expected and two were observed (p=0.21).  In female CD-1 mice and Swiss Albino mice, the expected and observed numbers are approximately equal.  However, in male CD-1 mice, there were 2.1 tumors expected for $p_{Trend} \leq 0.05$ or $p_{Hist} \leq 0.05$ and eight were observed (p<0.001) and there were 0.4 expected for $p_{Trend} \leq 0.01$ or $p_{Hist} \leq 0.01$ and five 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 4.6 expected responses for $p_{Trend} \leq 0.01$ or $p_{Hist} \leq 0.01$ and 12 observed (p<0.001).  Thus, chance does not explain the positive results seen in these studies.

**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.  Thus, either the studies are not good replicates so 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 five mouse studies that were of sufficient quality and with sufficient details available for inclusion in this evaluation.

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, kidney adenomas in male Swiss albino mice and hemangiomas in female Swiss albino mice.  Thus, glyphosate causes cancer in mammals.


**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)**[112] 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[113], but are only now being accepted as a means of evaluating literature in toxicological evaluations[114-117].

In this portion of the report, I am focusing on the mechanisms that can cause cancer. **Smith et al. (2015)**[37] 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 16 (copied from Table 1 of **Smith et al. (2015)**[37]).  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.

**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

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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 16:** Key characteristics of carcinogens, **Smith et al. (2016)**[37]

| 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.

**Genotoxicity in Humans *in-vivo***

Three studies have evaluated the potential genotoxicity of glyphosate formulations in exposed humans. **Paz-y-Miño et al. (2007)**[118] 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)**[119] 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)**[120] 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

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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)**[121] 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.

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.

### 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)**[122] 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)**[123] 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)**[124] 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)**[125] 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)**[126] 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)**[127] 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)**[128] 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)**[124] 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)**[129] 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)**[130] 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)**[124, 131] 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)**[132] 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.

**Genotoxicity in Non-Human Mammals (*in vivo*)**

**Bolognesi et al. (1997)**[130] 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)**[133] exposed groups of six (controls, lowest doses of glyphosate-salt and Roundup) or three Swiss CD-1 mice (males and females, specific numbers not

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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)**[134] 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)**[130] 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)**[124] 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)**[135] 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)**[136] 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)**[137] 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)**[138] 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)**[139] 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)**[140] 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)**[141] 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.

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

**Li and Long (1988)**[141] 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)**[142] 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)**[131] 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)**[143] 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)**[144] 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.

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

Four studies[123, 145-147] in fish have seen positive results for genotoxicity (DNA strand breaks, different assays) following exposure to glyphosate.  In addition, one study[148] in oyster sperm and embryos exposed to glyphosate saw no increase in DNA damage (comet assay) and one study[149] in two strains of Drosophila melanogaster showed an increase in mutations (wing spot test) at the higher doses of exposure.

Fourteen studies[137, 145, 147, 150-160] 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[150, 152] were negative for micronucleus formation after exposure to glyphosate formulations and one of these[150] was also negative for chromosomal aberrations but both were positive in other markers of genotoxicity.  Two studies[161, 162] demonstrated genotoxicity (DNA strand breaks, micronuclei) in caiman from *in-vivo* exposure to a glyphosate formulation. Three studies[163-165] demonstrated genotoxicity (DNA strand breaks, micronucleus formation) in frogs or tadpoles from exposure to glyphosate formulations.  One study[148] in oyster sperm and embryos, one study[166] in clams and one study[167] in mussels exposed to a glyphosate formulation saw no increase in DNA damage (comet assay).  One study[168] in snails saw increased DNA damage (comet assay) following exposure to a glyphosate formulation. Two studies[169, 170] in worms saw mixed results for DNA damage (comet assay) with one of these studies[169] showing a positive result for micronucleus formation. One study[171] in Drosophila melanogaster showed an increase in sex-linked recessive lethal mutations.

In the published literature, five studies evaluated the impact of glyphosate in *in vitro* systems.  Two of these studies[172, 173] 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[174] in eukaryote fish saw a significant increase in DNA strand breaks (comet assay) without S9 activation. Another study[141] showed no increase in reverse mutations in two strains of bacteria with and without S9 activation.

**Williams et al. (2000)**[175] 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[134] 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[141, 176] of glyphosate using reverse mutation assays are available

from the scientific literature, both of which are negative.


**Regulatory Studies**

**EFSA**[89] 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**[61] 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)**[177] 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**[89] 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**[61] 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)**[177] 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**[89] 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**[61] 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[124, 131, 143, 178] and are reviewed above. Surprisingly, EPA refers to the study by **Manas et al. (2009)**[124] as negative although it was clearly positive in the comet assay., Additionally, EPA refers to the study by **Sivikova and Dainovsky (2006)**[143] 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)**[177] 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[89].

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**EFSA**[89] 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**[61] (EPA Table 5.5) cites 20 micronucleus assays, four are available in the scientific literature and three are reviewed above (the fourth reference[179] 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)**[177] 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)**[177] 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[179] 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.

### 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, three studies in rats, nine studies in human lymphocytes and four 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 16 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 six studies in this category, five 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.

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 six studies using the comet assay were positive with no study showing a negative response below 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 four 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. Both 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.  While changes have been seen in three of the four 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 17:** 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 | 5 | 2(1) | 2 | |
| | | Hep 2 | 1 | | | |
| | | GM 38 HT1080 | 1 | | | |
| | | GM 5757 | 1 | | | |
| | | TR146 | 1 | | 1 | |
| *In vivo* | Swiss CD-1 Mouse | Liver/Kidney | 1 | 1 | 2 | |
| *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[89] and Kier and Kirkland (2000)[177]; [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 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)**[120] 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)**[180] 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)**[177] 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 2 is a reprint of Figure 1 from the study by **Ghisi et al. (2016)**[180] 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)**[180] 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 2:** Forest plot of studies evaluating micronucleus frequency in glyphosate exposure, arranged by effects size. 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)[180]. Grand Mean is the overall mean effects size of all studies. [Reprinted from Ghisi et al. (2016)[180]]
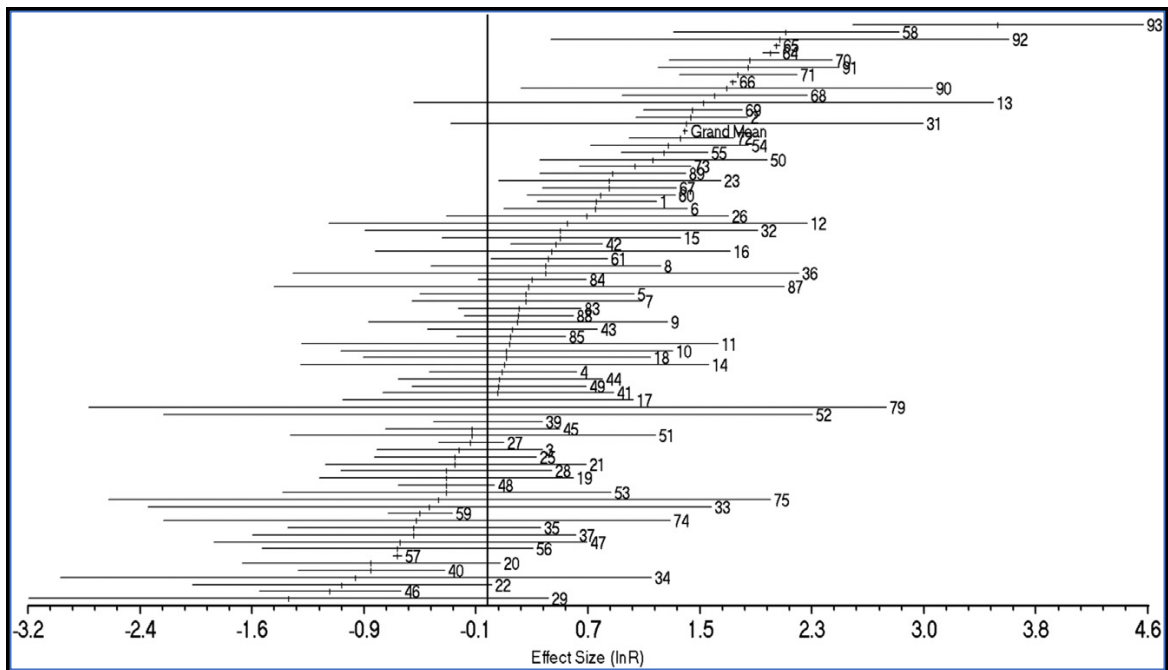


From a simply statistical perspective, there is another way in which one can decide if the positive findings in the micronucleus assays in the mice are due to chance. For the glyphosate studies, if one adds up all of the individual experimental groups, there are 79 total groups which correspond to 79 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

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are observed (p<0.01).  Now expanding to all 69 studies presented in Table 17, 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 69 experiments in Table 17, there were eight experiments from five research publications that addressed both glyphosate and a glyphosate formulation in the same laboratory.  Of these, two were negative for both glyphosate and the formulation and do not contribute to a discussion of relative potency.  The remaining six can provide some guidance on the relative potency of glyphosate to glyphosate formulations.  In **Koller et al. (2007)**[127], 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)**[133], 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. 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.

**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[181-186] including cancer[37, 187-191].  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.

**Oxidative Stress in Human Cells (*in vitro*)**

**Mladinic et al. (2009)**[122] 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)**[192] 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)**[193] 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<0.0-5) for the glyphosate formulation (increased SOD activity) but not for glyphosate alone.

**Coalova et al. (2014)**[194] 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)**[195] 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)**[196] 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[197, 198].

**George and Shukla (2013)**[199] 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[197, 198] that affect the clear

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interpretation of these results.

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

**Bolognesi et al. (1997)**[130] 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[200]. 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)**[139] 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)**[201] 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)**[202] 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[203], they demonstrate that lipoic acid eliminates or severely reduces the impacts of glyphosate on the brain.

**Cattani et al. (2014)**[204] 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)**[82] 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.

## 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)**[205]. 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[147, 156-159, 206-217].

## Summary for Oxidative Stress

Seven studies addressed oxidative stress in human cells and another six 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 Hep-G2 cells, no increased oxidative stress was seen for a single concentration of 900 µg/l. 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

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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.  In Swiss albino mice, topical application of a glyphosate formulation to the skin resulted in a proteomic fingerprint suggesting oxidative stress was increased.

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.

### 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.**

# Biological Gradient

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

**Eriksson et al. (2008)**[46]  divided their cases and controls into those with ≤10 days per year of exposure and those with >10 days per year of exposure. The ORs were calculated using a multivariate analysis that included agents with statistically significant increased OR, or with an OR > 1.50 and at least 10 exposed subjects.  ORs for glyphosate were 1.69 (0.70-4.07) for ≤10 days per year and 2.36 (1.04-5.37) for >10 days per year. In their multivariate analysis, latency periods of 1-10 years showed an OR of 1.11 (0.24-5.08) and >10 years had an OR of 2.26 (1.16-4.40).  Thus, they show an increase with intensity of exposure and with latency.

**McDuffie et al. (2001)**[50], using a conditional logistic regression analysis controlling for major chemical classes of pesticides and all other covariates with p<0.05, the OR for ≤2 days per year of exposure was 1.0 (0.63-1.57) and for >2 days per year, the OR was 2.12 (1.20-3.73). Thus, they show an increase with intensity of exposure.

**De Roos  et al. (2005)**[45] 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).   For exposure measurements b and c, they divided the respondents into tertiles chosen *a priori* to avoid having sparse data when dealing with rare tumors.  For cumulative exposure days and 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 adjusted for demographic and lifestyle factors and other pesticides (30,699 subjects).  When

intensity-weighted exposure days are examined, the RRs drop with values of 0.6 (0.3-1.1) and 0.8 (0.5-1.4) for tertiles 2 and 3, respectively adjusted for demographic and lifestyle factors and other pesticides (30,699 subjects).  Thus, they do not see a biological gradient in their responses.  However, the high frequency of exposure to many pesticides (e.g. 73.8% were exposed to 2,4-D) 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.

**Eriksson et al. (2008)**[46] and **McDuffie et al. (2001)**[50] 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).  It is not possible to resolve the remaining differences between these three 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.**

## 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.**

## Specificity

There are other causes of NHL[218-221] so this group of cancers is not specific to glyphosate. **There is little support for specificity**.

## Coherence

Humans, coming into contact with glyphosate, can absorb the compound into their bodies where it has been measured in blood and in urine[56, 222-226].  In laboratory animals, absorption, distribution and elimination of glyphosate and glyphosate compounds have been studied[140, 227] and show that glyphosate gets into the animal's 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[228-232].  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.

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

## 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.

## 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.

## Summary

Table 18 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

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causes malignant lymphomas, kidney adenomas in male Swiss albino mice and hemangiomas in female Swiss albino mice.  Thus, glyphosate causes cancer in mammals.  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 18: Summary conclusions for Hill's nine aspects of epidemiological data and related science

| Aspect | Conclusion | Reason |
|---|---|---|
| Consistency of the observed association | Strong | Multiple studies, all are positive, meta-analysis shows little heterogeneity, different research teams, different continents, different questionnaires, no obvious bias or confounding |
| Strength of the observed association | Strong | Six 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 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 | Not needed | NHL has other causes, this does not subtract from the causal argument |
| Coherence | Strong | Glyphosate is absorbed, distributed and excreted from the body, cancers seen in the mice have strong similarity to human NHL |
| 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 two 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.

NHL is not specific to glyphosate exposure.  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)**[36] 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.**

## 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)"*[56].

The IARC preamble[30] 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[34]).

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*

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*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 19 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, but I also evaluated, where possible, the proprietary data supporting the regulatory decisions.  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.
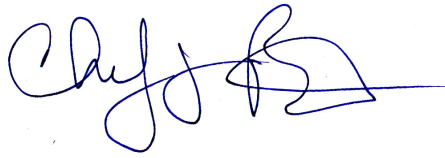
## The EPA Assessment of Glyphosate

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**[54]. 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 with 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 the evaluation done by the **European Food Safety Authority**[89] and the **European Chemical Agency**[233].  My detailed comments to these

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agencies on their risk assessments are attached.  There were comments to my comments to EPA by other scientists and I also responded to those comments in the EPA docket for glyphosate.  These are also included in the attached Appendices.

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Dr. Christopher J. Portier

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