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Research Article

Cancer Epidemiology, Biomarkers & Prevention

Comprehensive Evaluation of Medical Conditions Associated with Risk of Non-Hodgkin Lymphoma using Medicare Claims ("MedWAS")

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Abstract

Background: Certain medical conditions affect risk of non Hodgkin lymphoma (NHL), but the full range of associations is unknown. We implemented a novel method ("medical condition wide association study," MedWAS) to comprehensively evaluate medical risk factors for NHL documented in administrative health claims.

Methods: Using Surveillance, Epidemiology, and End Results (SEER) Medicare data, we conducted a case control study comparing NHL cases [*N* 52,691, age 66+ years, with five subtypes: chronic lymphocytic leukemia/small lymphocytic lymphoma, diffuse large B cell lymphoma (DLBCL), follicular lymphoma, marginal zone lymphoma (MZL), T cell lymphoma (TCL)] to controls (*N* 200,000). We systematically screened for associations with 5,926 medical conditions documented in Medicare claims more than 1 year before selection.

Results: Fifty five conditions were variously associated with NHL. Examples include well established associations of human immunodeficiency virus, solid organ transplantation, and hepatitis C virus with increased DLBCL risk (ORs 3.83, 4.27, and 1.74, respectively), and autoimmune conditions with DLBCL and MZL (e.g., ORs of 2.10 and 4.74, respectively, for Sjögren syndrome). Risks for all NHL subtypes were increased after diagnoses of nonmelanoma skin cancer (ORs 1.19 1.55), actinic keratosis (1.12 1.25), or hemolytic anemia (1.64 4.07). Nine additional skin conditions increased only TCL risk (ORs 2.20 4.12). Diabetes mellitus was associated with increased DLBCL risk (OR 1.09). Associations varied significantly across NHL subtypes for 49 conditions (89%).

Conclusion: Using an exploratory method, we found numerous medical conditions associated with NHL risk, and many associations varied across NHL subtypes.

Impact: These results point to etiologic heterogeneity among NHL subtypes. MedWAS is a new method for assessing the etiology of cancer and other diseases. *Cancer Epidemiol Biomarkers Prev; 25(7): 1105 13. ©2016 AACR.*

Introduction

Non Hodgkin lymphoma (NHL) is a common malignancy, with 465,000 incident cases worldwide in 2013 (1). Incidence rises with age, and 57% of U.S. cases occur after the age of 65 years (2). Although considered a single entity for descriptive purposes, NHL comprises a group of heterogeneous subtypes with distinct clinical presentations and, as is increasingly recognized, differing causal pathways (i.e., etiologic heterogeneity; ref. 3). Common NHL subtypes include tumors derived from B cells such as diffuse large B cell lymphoma (DLBCL), chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL), follicular lymphoma, and marginal zone lymphoma (MZL). T cell lymphomas (TCL) are less common.

Among the strongest risk factors for NHL are medical conditions, including those associated with immune dysfunction and chronic infections. For example, immunosuppression due to human immunodeficiency virus (HIV) infection or solid organ transplantation greatly increases NHL risk (4, 5). Immunosuppression facilitates activation of Epstein Barr virus infection, which contributes especially to DLBCL. Autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus, and Sjögren syndrome increase risk for DLBCL and MZL (6). These medical conditions are thought to promote the development of NHL by causing long term immune activation. Treatment of these conditions by immunosuppressive medications also likely contributes. In contrast, medical risk factors for CLL/SLL, follicular lymphoma, and TCL are not clearly established.

Large administrative databases provide a valuable resource for examining associations between medical risk factors and cancer. We have previously used the Surveillance, Epidemiology, and End Results (SEER) Medicare database (described below) to conduct case control studies among the U.S. elderly population, assessing associations between various medical conditions and cancers such as NHL, leukemias, and skin cancers (7 10). Strengths of SEER Medicare include availability of data from cancer registries (which provide reliable case ascertainment and detailed

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Note: Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (http://cebp.aacrjournals.org/).

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information on cancer subtypes), its large size (i.e., 1.6 million cancer cases diagnosed in 1991 2009), and information on medical risk factors detailed in Medicare claims beginning at the age of 65 years (11). Until now, SEER Medicare has been used to evaluate a limited number of medical risk factors for cancer (7 10, 12 15), selected on the basis of previously published findings or plausible biologic mechanisms.

A comprehensive assessment of medical conditions in associ ation with cancer would be an attractive new approach to char acterizing a wide spectrum of risk factors. Using claims data, for example, this could be done by separately evaluating associations with every medical condition specified by billing codes. We term this novel approach "MedWAS," for "medical condition wide association study," given its use of a broadly agnostic assessment that is a feature of genome wide association studies (GWAS). One might anticipate that a MedWAS assessment of all medical con ditions could uncover previously unsuspected associations with cancer, which would then prompt an investigation into possible biologic mechanisms. A comprehensive assessment might also uncover a diversity of conditions associated with different cancer subtypes, providing evidence for etiologic heterogeneity.

In the current study, we implement this new MedWAS approach using SEER Medicare data to assess potential risk factors for five subtypes of NHL. We demonstrate the utility of this method for characterizing the spectrum of medical risk factors for NHL, assessing etiologic heterogeneity among NHL subtypes, and identifying new medical conditions associated with NHL. Given the large multiplicity of testing, we emphasize the explor atory nature of this approach. We review possible etiologic and artifactual explanations for associations that we uncover.

Materials and Methods

Subject selection and ascertainment of medical conditions

SEER Medicare (http://healthcaredelivery.cancer.gov/seerme dicare/) links data from SEER cancer registries (covering 28% of the U.S. population in 2010) and Medicare (which provides medical insurance for the U.S. elderly; ref. 11). For this study, we selected cases and controls from the SEER Medicare dataset as described previously (16). Specifically, we identified NHL cases in SEER that were indicated by SEER to be the person's first invasive cancer (except for a possible diagnosis of basal and squamous cell skin cancers, which are common nonmelanoma skin cancers not captured by SEER). We included the five most common NHL subtypes, defined according to the World Health Organization classification (17): CLL/SLL, DLBCL, follicular lymphoma, MZL, and TCLs considered as a group.

All Medicare beneficiaries are enrolled in part A, which covers hospital care, and most also subscribe to part B which covers physician and outpatient services. Health maintenance organiza tions (HMO) do not routinely bill Medicare for individual encounters. To ensure availability of Medicare claims prior to NHL diagnosis, we required cases that: (i) were ages 66 99 years at diagnosis; (ii) were diagnosed in 1992 2009; (iii) had a min imum of 13 months of part A and part B Medicare coverage before diagnosis, during which they were not enrolled in an HMO; and (iv) had at least one Medicare claim for a hospi talization (documented in the MEDPAR file), provider visit (NCH file), or outpatient services (OUTPATIENT file) at least 13 months before diagnosis. Medicare coverage and claims were considered back to the later of age 65 or a calendar year cutoff that varied according to calendar year of NHL diagnosis based on the availability of Medicare claims data (1991 for cases diagnosed in 1992 2002, 1998 for 2003 2005, 2000 for 2006 2007, 2002 for 2008 2009). Cases diagnosed only on autopsy or death certificate were excluded. Selection yielded N 52,691 cases. For comparison, omission of the requirements for non HMO coverage and at least one Medicare claim would have yielded N 68,044 cases.

Controls were selected from the random 5% sample of Medi care beneficiaries living in SEER areas included in the SEER Medicare dataset (16). Controls were selected separately for each calendar year 1992 2009. As of July 1 (the selection date) of each year, controls were required to: (i) be alive and cancer free; (ii) have at least 13 months of prior Medicare coverage; and (iii) have at least one Medicare claim at least 13 months earlier; age and calendar year cutoffs, and requirements for Medicare coverage and claims, were as described above for cases. From eligible controls, we randomly selected 200,000 controls, frequency matched to cases according to calendar year, sex, age, and race. Controls could be selected more than once for multiple years or included later as a case.

We searched Medicare claims to identify medical conditions diagnosed more than 12 months before case/control selection. The 1 year period immediately before case/control selection was excluded to minimize bias due to reverse causality or differential medical work up of cases. We initially considered medical con ditions defined by the first four digits of International Classifica tion of Diseases (version 9, ICD 9) codes. However, we also considered three digit codes when providers only indicated this level of detail. To indicate that the condition was present, we required one inpatient claim with the diagnosis (MEDPAR file) or at least two physician or outpatient claims at least 30 days apart (NCH and OUTPATIENT files). Medical conditions could be described at any position in the claim, that is, as primary or secondary diagnoses.

Statistical analysis

ICD 9 is a hierarchical coding system designed to provide an international standard for morbidity and mortality statistics, and especially as implemented in Medicare claims, for use in reim bursement of care providers. One challenge is that no level of the scheme uniformly captures all medical conditions at the same degree of detail, and some conditions are indicated by multiple codes in separate parts of the classification. We therefore used a stepwise approach to identify medical conditions associated with NHL subtypes (Fig. 1).

Specifically, in the first step, the prevalence of every ICD 9 specified condition was compared separately between each NHL subtype and all controls. This group of unselected conditions (SELECT0) was defined by categorizing Medicare claims based on all provided four digit ICD 9 codes (or occasionally, as noted above, by three digit codes). In comparing the prevalence of the conditions in cases and controls, we selected conditions for further evaluation if: (i) the lowest achievable significance level computed from marginal totals in the 2×2 table (minalpha statistic) was less than 0.001 (18); and (ii) the P value from the Cochran Mantel Haenszel test (conditioning on the matching factors) was less than the Bonferroni cutoff (defined as 0.05 divided by the number of conditions remaining after applying the minalpha criterion). We excluded ICD 9 coded conditions for invalid conditions, specifically those obviously corresponding

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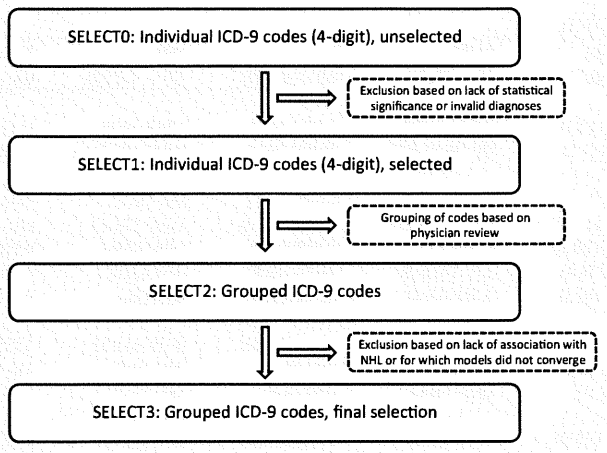

Figure 1.
Stepwise selection of medical conditions associated with subtypes of non Hodgkin lymphoma (NHL). The figure illustrates the steps used to identify a final group of medical conditions, defined by ICD 9 codes, that were associated with each NHL subtype.

to a possible NHL diagnosis (e.g., NHL itself, lymphadenopathy, or splenomegaly), nonspecific symptoms (e.g., headache, fatigue), and spurious codes that could not be matched to diagnoses. Because cases and controls were selected to have no prior SEER documented cancer, we considered claims for previ ous cancer diagnoses (other than nonmelanoma skin cancer) as having uncertain reliability; therefore, we also excluded these claims diagnoses from analysis.

This procedure yielded a subset of conditions for each NHL subtype, which we refer to as SELECT1. Next, we used binary logistic regression models to derive ORs, measuring the associa tions of each SELECT1 condition with the NHL subtype, adjusted for demographic characteristics (sex, age, and calendar year of case/control selection, race), and as a measure of health care utilization, the number of provider claims per year (see Table 1 footnote for details). Each NHL subtype was compared with all controls, and the variance of the ORs accounted for the multiple sampling of some controls (16).

Two physicians reviewed these results to group similar SELECT1 conditions together (e.g., multiple ICD 9 codes for skin cancer at different body sites), add related ICD 9 codes not in SELECT1 as part of the groups, and in rare instances, to break SELECT1 conditions into finer categories (e.g., distinguishing different hepatitis virus infections based on five digit codes). SELECT1 conditions were removed if they were rare (<5 affected cases when the OR > 1, or <100 affected controls when the OR < 1) and could not be grouped with other conditions. This process led to SELECT2 conditions for each NHL subtype.

We again used binary logistic regression to assess associations of SELECT2 conditions, adjusted for demographic characteristics and yearly physician claims. We excluded SELECT2 conditions for which the adjusted OR was both nonsignificant ($P \geq 0.05$) and close to the null value, or for which the models did not converge due to small numbers of affected cases or controls. This process yielded the final group of SELECT3 medical conditions associated with each NHL subtype.

We compiled the list of SELECT3 medical conditions across the five NHL subtypes and used polytomous logistic regression to assess the association of each SELECT3 condition with each subtype, adjusting for demographic characteristics and yearly provider claims. Although we present ORs for each condition with all five subtypes, we focus on the subset of conditions that were associated with each subtype in its separate SELECT3 anal ysis. Each polytomous logistic regression model provided a test of heterogeneity of the ORs across NHL subtypes.

We also conducted a sensitivity analysis for SELECT3 condi tions to further minimize the possibility of reverse causality. Specifically, we excluded from evaluation the 3 year period immediately preceding NHL diagnosis/control selection. This approach left *N* 39,995 cases and *N* 158,706 controls (age 68 or older) with evaluable time covered by Medicare. For these subjects, we reascertained the previously identified SELECT3 medical conditions, this time excluding the 3 year window before case/control selection, and reran the polytomous logistic regres sion models.

Replication of selected findings

We sought to replicate selected associations with SELECT3 medical conditions in two independent datasets: the National Institutes of Health AARP Diet and Health Study (NIH AARP) and the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial (PLCO), as described in the notes to Supplementary Table S1. These replications required data on both the medical condi tion and NHL subtypes, so replications could not be undertaken for all findings. We utilized a two sided α of 0.05 to assess significance in these analyses. In some instances, the magnitude of the association that we tried to replicate and the number of cases with each NHL subtype were both small, greatly limiting the statistical power for the replication (Supplementary Table S1).

Results

Characteristics of study subjects and stepwise selection of medical conditions

We included 52,691 cases with NHL (*N* 19,078 for DLBCL, *N* 18,236 for CLL/SLL, *N* 8,881 for follicular lymphoma, *N* 4,289 for MZL, *N* 2,207 for TCL) from SEER Medicare. Overall, cases were well matched to the 200,000 controls according to demographic characteristics, although there were some minor differences among NHL subtypes (Table 1). Cases tended to have slightly shorter duration of prior Medicare coverage (median 52 vs. 54 months, excluding the 12 months immediately before case/ control selection) but had more physician visits per year (Table 1).

Figure 1 and Table 2 document the process by which medical conditions associated with each NHL subtype were identified. For each NHL subtype, we screened 5,605 to 5,785 conditions indi cated by unique four digit (and occasional three digit) ICD 9 codes, or a total of 5,926 conditions across all five subtypes (SELECT0 conditions). More than 97% of these conditions were excluded because they were not significantly associated with the subtype or were for invalid diagnoses. This procedure left 30 to 52 remaining conditions for each subtype, identified by (mostly) four digit ICD 9 codes (SELECT1 conditions; see Supplementary Table S2 for a complete list). Two physicians reviewed these conditions and grouped related codes to create 13 to 28 SELECT2 conditions for each NHL subtype.

Most SELECT2 conditions remained associated with their respective NHL subtypes in multivariate logistic regression mod els, yielding the final SELECT3 group of medical conditions (*N* 17 conditions for CLL/SLL, *N* 27 for DBLCL, *N* 13 for

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Table 1. Characteristics of NHL cases and controls in SEER Medicare (1992 2009)

Characteristic, *N* (%)	Controls (*N* = 200,000)	NHL cases combined (*N* = 52,691)	CLL/SLL (*N* = 18,236)	DLBCL (*N* = 19,078)	FL (*N* = 8,881)	MZL (*N* = 4,289)	TCL (*N* = 2,207)
Sex							
Male	95,262 (47.6)	25,096 (47.6)	9,675 (53.1)	8,640 (45.3)	3,828 (43.1)	1,748 (40.8)	1,205 (54.6)
Female	104,738 (52.4)	27,595 (52.4)	8,561 (47.0)	10,438 (54.7)	5,053 (56.9)	2,541 (59.2)	1,002 (45.4)
Age in years at case/control selection							
65 69	30,397 (15.2)	8,008 (15.2)	2,688 (14.7)	2,578 (13.5)	1,685 (19.0)	691 (16.1)	366 (16.6)
70 74	48,228 (24.1)	12,706 (24.1)	4,242 (23.3)	4,425 (23.2)	2,404 (27.1)	1,025 (23.9)	610 (27.6)
75 79	49,090 (24.6)	12,934 (24.6)	4,430 (24.3)	4,784 (25.1)	2,168 (24.4)	1,012 (23.6)	540 (24.5)
80 84	39,579 (19.8)	10,428 (19.8)	3,531 (19.4)	3,973 (20.8)	1,622 (18.3)	902 (21.0)	400 (18.1)
85+	32,706 (16.4)	8,615 (16.4)	3,345 (18.3)	3,318 (17.4)	1,002 (11.3)	659 (15.4)	291 (13.2)
Race							
White	178,689 (89.3)	47,080 (89.4)	16,679 (91.5)	16,701 (87.5)	8,160 (91.9)	3,751 (87.5)	1,789 (81.1)
Black	9,267 (4.6)	2,439 (4.6)	1,027 (5.6)	694 (3.6)	292 (3.3)	208 (4.9)	218 (9.9)
Other	12,044 (6.0)	3,172 (6.0)	530 (2.9)	1,683 (8.8)	429 (4.8)	330 (7.7)	200 (9.1)
Duration of Medicare coverage in months at case/control selection[a]							
1 24	37,880 (18.9)	11,936 (22.7)	4,352 (23.9)	4,101 (21.5)	2,184 (24.6)	803 (18.7)	496 (22.5)
25 49	50,070 (25.0)	12,759 (24.2)	4,612 (25.3)	4,441 (23.3)	2,194 (24.7)	966 (22.5)	546 (24.7)
50 74	62,543 (31.3)	17,681 (33.6)	5,898 (32.3)	6,533 (34.2)	2,885 (32.5)	1,620 (37.8)	745 (33.8)
75+	49,507 (24.8)	10,315 (19.6)	3,374 (18.5)	4,003 (21.0)	1,618 (18.2)	900 (21.0)	420 (19.0)
Average number of physician visits per year prior to case/control selection[b]							
0	56,079 (28.0)	2,921 (5.5)	1,143 (6.3)	1,065 (5.6)	483 (5.4)	107 (2.5)	123 (5.6)
0.001 3.124	43,807 (21.9)	12,862 (24.4)	4,413 (24.2)	4,799 (25.2)	2,265 (25.5)	926 (21.6)	459 (20.8)
3.125 7.499	49,969 (25.0)	17,304 (32.8)	5,921 (32.5)	6,248 (32.8)	2,996 (33.7)	1413 (32.9)	726 (32.9)
7.500+	50,145 (25.1)	19,604 (37.2)	6,759 (37.1)	6,966 (36.5)	3,137 (35.3)	1,843 (43.0)	899 (40.7)

Abbreviation: FL, follicular lymphoma.

[a]Medicare coverage refers to part A and part B coverage, without enrollment in a health maintenance organization. The coverage in the 12 months prior to NHL diagnosis/control selection is not counted in this total.

[b]Physician visits are for the period excluding the 12 months prior to case/control selection. This variable is based on a count of records in the NCH file that have the following Healthcare Common Procedure Coding System (HCPCS) codes: 99201 99255, 99261 99263, 99271 99275, 99301 99340, 99341 99353, 99354 99357, 99381 99429. The count excludes records for radiology, anesthesiology, and pathology providers, and includes a maximum of one NCH record per day.

follicular lymphoma, *N* 18 for MZL, and *N* 15 for TCL, for a total of *N* 55 unique SELECT3 conditions; Table 2). ICD 9 codes for SELECT3 conditions, as well as the number of subjects with each condition, are presented in Supplementary Table S3.

Findings of MedWAS analyses

Table 3 presents associations for the 55 SELECT3 medical conditions with each NHL subtype. Of note, most conditions (*N* 49, 89%) varied significantly in their associations across NHL subtypes (i.e., $P_{heterogeneity} < 0.05$; Table 3).

We highlight associations for each subtype that were iden tified in the SELECT3 group for that subtype (shaded in gray in Table 3). For most of these, the 95% confidence interval (CI) for the OR excludes 1.00 (underlined in Table 3). Only 3 of the 55 medical conditions were associated with increased risk for all five NHL subtypes: nonmelanoma skin cancer (ORs 1.19 1.55), actinic keratosis (1.12 1.25), and hemolytic anemia (1.64 4.07; Table 3).

Among the positive associations, several immunodeficiency or infectious conditions were associated with increased risk for 1 2 NHL subtypes (Table 3). Specifically, associations were observed for HIV infection and solid organ transplantation with DLBCL (ORs 3.83 and 4.27, respectively), and deficiency of humoral immunity with CLL/SLL (3.85) and TCL (5.70). CLL/SLL risk was increased in association with herpes zoster (OR 1.44), acute sinusitis (1.12), and acute bronchitis (1.06), and DLBCL was increased with hepatitis C virus (HCV) infection (1.74).

Autoimmune diseases were also associated with increased risk for some subtypes, including for DLBCL with rheumatoid arthritis (OR 1.43), sarcoidosis (2.11), and uveitis (3.17). Systemic lupus

Table 2. Number of evaluated medical conditions at each stage of analysis of SEER Medicare claims data

Analysis stage	Description	Number of conditions, by NHL subtype					Number of unique conditions across all subtypes
		CLL/SLL	DLBCL	FL	MZL	TCL	
SELECT0	Individual ICD 9 codes, unselected	5,762	5,785	5,712	5,618	5,605	5,926
SELECT1	Individual ICD 9 codes, selected for statistical significance according to multiple testing procedure, excluding invalid conditions	30	43	27	50	52	146
SELECT2	Grouped ICD 9 codes, based on review by physicians	17	28	13	18	18	59
SELECT3	Grouped ICD 9 codes, excluding conditions that were not associated with NHL subtype or for which models did not converge	17	27	13	18	15	55

Abbreviation: FL, follicular lymphoma.

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Table 3. ORs associating SELECT3 medical conditions with NHL, SEER Medicare (1992–2009)

	Adjusted OR (95%CI)[a]					
Condition	**CLL/SLL**	**DLBCL**	**FL**	**MZL**	**TCL**	**$P_{Heterogeneity}$**
Immunodeficiency related conditions						
HIV	0.86 (0.34–2.16)	3.83 (2.28–6.43)	0.40 (0.06–2.90)	2.15 (0.66–6.96)	0	<0.0001
Deficiency of humoral immunity	3.85 (2.43–6.11)	1.42 (0.72–2.81)	0.57 (0.14–2.31)	2.67 (1.05–6.75)	5.70 (2.29–14.18)	0.0058
Solid organ transplantation	0.91 (0.53–1.55)	4.27 (3.23–5.64)	0.95 (0.47–1.95)	1.92 (0.97–3.79)	3.58 (1.82–7.03)	<0.0001
Infections						
Herpes zoster	1.44 (1.28–1.61)	1.23 (1.10–1.39)	1.04 (0.86–1.25)	1.42 (1.15–1.76)	1.20 (0.85–1.68)	0.0244
HCV	0.61 (0.41–0.92)	1.74 (1.37–2.22)	0.62 (0.36–1.08)	1.27 (0.77–2.10)	0.62 (0.23–1.67)	<0.0001
Acute sinusitis	1.12 (1.05–1.20)	0.98 (0.92–1.05)	1.12 (1.02–1.22)	1.10 (0.97–1.24)	0.95 (0.79–1.15)	0.0170
Acute bronchitis	1.06 (1.01–1.11)	0.94 (0.90–0.99)	0.94 (0.88–1.02)	1.02 (0.92–1.12)	0.96 (0.84–1.11)	0.0069
Chronic bronchitis	0.82 (0.77–0.89)	0.79 (0.75–0.84)	0.79 (0.71–0.88)	0.95 (0.83–1.08)	0.73 (0.60–0.89)	0.0719
Urinary tract infection	0.89 (0.85–0.93)	0.83 (0.80–0.87)	0.83 (0.78–0.88)	0.79 (0.72–0.86)	0.77 (0.68–0.88)	0.0279
Autoimmune and inflammatory conditions						
Rheumatoid arthritis	0.90 (0.82–1.00)	1.43 (1.32–1.55)	1.20 (1.07–1.36)	1.18 (1.01–1.39)	1.31 (1.05–1.64)	<0.0001
Systemic lupus erythematosus	0.82 (0.60–1.14)	1.74 (1.39–2.17)	1.06 (0.72–1.55)	2.57 (1.84–3.58)	2.25 (1.34–3.77)	<0.0001
Sjogren syndrome	1.05 (0.82–1.33)	2.10 (1.77–2.49)	1.57 (1.20–2.06)	4.74 (3.81–5.89)	1.78 (1.08–2.93)	<0.0001
Sarcoidosis	0.84 (0.50–1.39)	2.11 (1.50–2.96)	0.92 (0.47–1.81)	2.41 (1.37–4.24)	0.76 (0.19–3.07)	0.0033
Celiac disease	1.41 (0.84–2.34)	1.34 (0.81–2.23)	0.98 (0.43–2.23)	2.43 (1.19–4.96)	8.09 (4.36–15.02)	<0.0001
Uveitis	1.62 (0.85–3.09)	3.17 (1.97–5.08)	1.46 (0.58–3.64)	1.15 (0.28–4.73)	0	<0.0001
Elevated sedimentation rate	1.03 (0.79–1.36)	1.31 (1.03–1.66)	0.76 (0.49–1.19)	2.00 (1.39–2.86)	1.33 (0.68–2.58)	0.0074
Hematologic conditions						
Hemolytic anemia	3.60 (2.70–4.82)	1.99 (1.39–2.85)	1.64 (0.94–2.85)	4.07 (2.51–6.60)	2.97 (1.37–6.41)	0.0041
Aplastic anemia	1.49 (1.21–1.82)	1.37 (1.12–1.68)	1.13 (0.82–1.58)	3.26 (2.51–4.22)	1.20 (0.68–2.13)	<0.0001
Anemia NOS	1.01 (0.97–1.06)	1.03 (0.98–1.07)	0.86 (0.80–0.92)	1.12 (1.04–1.22)	0.97 (0.86–1.10)	<0.0001
Thrombocytopenia	1.73 (1.56–1.92)	1.46 (1.30–1.63)	1.01 (0.83–1.23)	2.21 (1.84–2.64)	1.48 (1.10–1.99)	<0.0001
Neutropenia	1.33 (1.11–1.61)	1.40 (1.18–1.67)	1.25 (0.95–1.64)	2.47 (1.91–3.18)	1.63 (1.05–2.52)	0.0004
Monoclonal paraproteinaemia	2.01 (1.56–2.59)	1.80 (1.39–2.34)	1.45 (0.96–2.20)	3.43 (2.40–4.91)	1.51 (0.71–3.19)	0.0095
Cryoglobulinemia	4.58 (1.05–20.03)	6.36 (1.62–25.03)	10.14(2.52–40.68)	10.23(1.80–58.14)	6.64 (0.57–76.77)	0.6777
Cardiovascular disorders						
Benign/unspecified hypertension	0.83 (0.80–0.86)	0.93 (0.89–0.96)	0.87 (0.83–0.92)	0.80 (0.74–0.86)	0.85 (0.77–0.94)	0.0001
Systolic heart failure	0.89 (0.85–0.94)	0.92 (0.88–0.96)	0.80 (0.74–0.86)	0.70 (0.63–0.78)	0.77 (0.67–0.88)	<0.0001
Abdominal aneurysm	0.67 (0.58–0.77)	1.05 (0.93–1.19)	0.84 (0.68–1.02)	0.92 (0.71–1.20)	0.88 (0.61–1.26)	0.0001
Endocrine/metabolic conditions						
Diabetes mellitus	0.96 (0.92–1.00)	1.09 (1.05–1.15)	0.89 (0.84–0.94)	0.77 (0.71–0.83)	0.93 (0.84–1.04)	<0.0001
Hyperlipidemia NOS	0.86 (0.83–0.90)	0.96 (0.92–0.99)	0.97 (0.92–1.01)	0.85 (0.80–0.91)	0.89 (0.81–0.98)	0.0000
Testicular hypofunction	0.89 (0.73–1.09)	0.90 (0.73–1.11)	1.10 (0.84–1.45)	1.58 (1.15–2.17)	0.76 (0.43–1.35)	0.0175
Neurological/psychiatric disorders						
Dementia, senile, or other causes	0.61 (0.56–0.66)	0.44 (0.40–0.48)	0.40 (0.35–0.47)	0.41 (0.34–0.49)	0.48 (0.37–0.62)	<0.0001
Arteriosclerotic dementia	0.54 (0.44–0.67)	0.35 (0.27–0.45)	0.32 (0.21–0.49)	0.33 (0.19–0.55)	0.50 (0.26–0.93)	0.0312
Parkinson disease	0.74 (0.65–0.85)	0.67 (0.59–0.77)	0.71 (0.58–0.87)	0.56 (0.41–0.75)	0.62 (0.42–0.91)	0.3946
Stroke, including TIA	0.77 (0.74–0.81)	0.86 (0.83–0.91)	0.82 (0.76–0.88)	0.74 (0.67–0.81)	0.79 (0.69–0.90)	0.0032
Depression NOS	0.82 (0.76–0.87)	0.74 (0.69–0.80)	0.70 (0.63–0.78)	0.71 (0.62–0.81)	0.85 (0.70–1.03)	0.0519
Psychosis NOS	0.64 (0.56–0.74)	0.47 (0.40–0.56)	0.53 (0.38–0.74)	0.37 (0.26–0.53)	0.43 (0.27–0.70)	<0.0001
Nonpsychotic mental disorder NOS	0.55 (0.41–0.73)	0.23 (0.15–0.35)	0.29 (0.16–0.54)	0.16 (0.05–0.50)	0.67 (0.32–1.42)	0.0021
Alcoholism	0.79 (0.68–0.92)	0.59 (0.50–0.71)	0.60 (0.46–0.78)	0.81 (0.59–1.11)	0.98 (0.68–1.42)	0.0235
Skin diseases						
Nonmelanoma skin cancer	1.19 (1.13–1.25)	1.20 (1.15–1.26)	1.25 (1.17–1.34)	1.24 (1.13–1.35)	1.55 (1.37–1.76)	0.0018
Actinic keratosis	1.12 (1.07–1.17)	1.14 (1.09–1.19)	1.20 (1.13–1.28)	1.17 (1.08–1.28)	1.25 (1.11–1.41)	0.1607
Atopic dermatitis	0.95 (0.79–1.13)	1.12 (0.96–1.30)	0.94 (0.73–1.21)	1.28 (0.97–1.70)	4.12 (3.25–5.22)	<0.0001
Contact dermatitis and other eczema	0.91 (0.86–0.96)	1.04 (0.99–1.10)	0.98 (0.91–1.06)	1.09 (0.98–1.20)	2.61 (2.33–2.93)	<0.0001
Dermatitis due to substances taken internally	0.93 (0.71–1.20)	0.94 (0.73–1.21)	0.75 (0.50–1.14)	1.29 (0.85–1.96)	4.05 (2.84–5.79)	<0.0001
Bullous skin diseases	0.97 (0.64–1.48)	1.14 (0.78–1.67)	1.10 (0.61–1.97)	0.81 (0.33–1.98)	3.43 (1.82–6.45)	0.0113
Discoid lupus	0.35 (0.16–0.79)	1.93 (1.35–2.76)	0.55 (0.23–1.35)	1.19 (0.52–2.70)	4.00 (2.04–7.85)	<0.0001
Psoriasis and similar disorders	0.82 (0.71–0.95)	1.25 (1.11–1.40)	0.98 (0.82–1.19)	1.34 (1.07–1.67)	3.72 (3.05–4.55)	<0.0001
Seborrheic keratosis	1.01 (0.95–1.07)	1.00 (0.95–1.06)	1.18 (1.09–1.28)	1.24 (1.12–1.37)	1.25 (1.08–1.46)	<0.0001
Folliculitis and related conditions	1.00 (0.78–1.29)	1.10 (0.85–1.41)	0.54 (0.32–0.89)	1.25 (0.79–1.97)	2.65 (1.73–4.07)	<0.0001
Asteatosis	0.86 (0.72–1.02)	0.98 (0.84–1.15)	1.00 (0.79–1.26)	1.07 (0.80–1.43)	2.20 (1.62–2.97)	<0.0001
Urticaria	0.96 (0.78–1.18)	1.01 (0.84–1.22)	1.15 (0.89–1.50)	0.97 (0.67–1.40)	2.42 (1.71–3.41)	<0.0001
Miscellaneous conditions						
Benign prostate hyperplasia	1.04 (0.99–1.10)	0.97 (0.91–1.03)	1.10 (1.02–1.20)	1.30 (1.15–1.46)	0.95 (0.82–1.10)	<0.0001
Gastric ulcer	0.92 (0.81–1.04)	0.88 (0.78–1.00)	0.88 (0.72–1.06)	1.55 (1.28–1.88)	0.84 (0.59–1.20)	<0.0001
Decubitus ulcer	0.76 (0.66–0.88)	0.57 (0.48–0.67)	0.56 (0.43–0.72)	0.48 (0.34–0.68)	0.64 (0.41–0.98)	0.0183
Specified anomalies of spinal cord (myelodysplasia)	3.20 (2.06–4.98)	1.69 (0.97–2.94)	0.57 (0.14–2.35)	2.23 (0.89–5.61)	1.93 (0.48–7.81)	0.0912
Asphyxia	0.90 (0.78–1.04)	0.79 (0.68–0.92)	0.52 (0.40–0.69)	0.60 (0.43–0.83)	1.00 (0.68–1.46)	0.0020
Hip fracture	0.76 (0.66–0.86)	0.62 (0.54–0.71)	0.50 (0.39–0.64)	0.71 (0.55–0.92)	0.61 (0.40–0.95)	0.0286

Abbreviations: FL, follicular lymphoma; TIA, transient ischemic attack.

[a]ORs are adjusted for sex, age, and calendar year of case/control selection, race, and number of provider claims per year. Results are not shown for models that failed to converge. ORs that are shaded were identified as SELECT3 conditions for the indicated NHL subtype, and underlining indicates statistical significance ($P < 0.05$).

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erythematosus was associated with risk of DLBCL (OR 1.74) and MZL (2.57); Sjögren syndrome with DLBCL (2.10) and MZL (4.74); and celiac disease with TCL (8.09). An elevated erythrocyte sedimentation rate was associated with increased risk of MZL (OR 2.00).

Among hematologic conditions (in addition to hemolytic anemia which was associated with all NHL subtypes), thrombo cytopenia was associated with increased risk for all subtypes other than follicular lymphoma (ORs 1.46 2.21). Aplastic anemia, anemia not otherwise specified (NOS), and neutropenia were positively associated with MZL (ORs 3.26, 1.12, and 2.47, respec tively). Monoclonal paraproteinemia was associated with CLL/ SLL, DLBCL, and MZL (ORs 1.80 3.43), and cryoglobulinemia with DLBCL (6.36) and follicular lymphoma (10.14).

Among skin conditions other than nonmelanoma skin cancer and actinic keratosis, nine were associated with increased risk only for TCL: atopic dermatitis (OR 4.12), contact dermatitis (2.61), dermatitis due to substances taken internally (4.05), bullous skin diseases (3.43), discoid lupus (4.00), psoriasis (3.72), folliculitis (2.65), asteatosis (2.20), and urticaria (2.42). Seborrheic keratosis was associated with increased risk of follicular lymphoma (OR 1.18) and MZL (1.24).

There were a few additional positive associations. Diabetes mellitus was associated with DLBCL (OR 1.09). Testicular hypo function, benign prostatic hyperplasia, and gastric ulcer were associated with MZL (ORs 1.58, 1.30, and 1.55, respectively), and spinal cord anomalies was associated with CLL/SLL (3.20).

No medical condition was inversely associated with all five NHL subtypes. However, inverse associations were observed with several neurologic/psychiatric conditions (Table 3). Three such conditions were inversely associated with all NHL subtypes except TCL: senile dementia (ORs 0.40 0.61), stroke (ORs 0.74 0.86), and psychosis NOS (0.37 0.64). Inverse associations were also observed for arteriosclerotic dementia with CLL/SLL, DLBCL, and follicular lymphoma (ORs 0.32 0.54); Parkinson disease with DLBCL (0.67); depression NOS with DLBCL (0.74) and follicular lymphoma (0.70); nonpsychotic mental disorder NOS with DLBCL (0.23); and alcoholism with DLBCL (0.59).

Among infections, inverse associations were found for chronic bronchitis and urinary tract infection with DLBCL (ORs 0.78 and 0.83, respectively). CLL/SLL was reduced in association with hypertension (OR 0.83), abdominal aortic aneurysm (0.67), and hyperlipidemia (0.86), and follicular lymphoma was reduced in association with systolic heart failure (0.80). Among miscella neous conditions, inverse associations were observed between decubitus ulcer and DLBCL (OR 0.57); asphyxia and follicular lymphoma (0.52); and hip fracture and DLBCL (0.62) and follicular lymphoma (0.50).

Sensitivity and replication analyses

We performed a sensitivity analyses for the 55 SELECT3 med ical conditions, excluding claims during the 3 years immediately before case/control selection. ORs were very similar to the primary analysis (Supplementary Table S4).

Table 4 presents results of replication analyses in additional populations for some SELECT3 conditions. For diabetes mellitus and DLBCL, positive associations in the NIH AARP and PLCO cohorts appeared consistent with the MedWAS observation, although the replications did not reach statistical significance. There was also an inverse (though statistically nonsignificant) association in PLCO between chronic bronchitis and DLBCL. Associations with hypertension and stroke were not significant in replication analyses.

Discussion

We surveyed a large number of medical conditions as risk factors for NHL using a new approach termed "MedWAS." This method characterized the full spectrum of medical conditions related to NHL among the U.S. elderly. Some associations that we document are well established, whereas others are new or less supported.

Importantly, for the 55 medical conditions retained in our final (SELECT3) analyses, we found that most associations varied significantly across the five NHL subtypes. In fact, most medical conditions were associated with only one or a few NHL subtypes. Because some associations are likely etiologic (as we review below), these differences point to etiologic heterogeneity, that is, distinct causal factors that contribute to each NHL subtype. Other differences in associations with environmental risk factors and genetic polymorphisms (3, 19 22) likewise support that different NHL subtypes arise through separate (although perhaps overlapping) mechanisms.

Some associations that we demonstrated reflect well estab lished contributions of chronic immune disturbances to devel opment of NHL. These include associations of HIV infection, HCV infection, and solid organ transplantation with DLBCL and, to a lesser extent, MZL (4, 5, 23). As described previously (6), we found an increased risk of DLBCL and/or MZL associated with a range of autoimmune conditions including rheumatoid arthritis, systemic lupus erythematosus, and Sjögren syndrome, and strongly ele vated risk of TCL with celiac disease. In striking contrast, none of these conditions was associated with CLL/SLL or follicular lym phoma. Increased risk of CLL/SLL following herpes zoster, acute sinusitis, and acute bronchitis may plausibly be a manifestation of chronic immune deficits preceding this malignancy (24, 25).

Interestingly, risk for each NHL subtype was elevated following nonmelanoma skin cancer. A few prior studies have described increased risk for NHL or CLL/SLL following diagnoses of basal or squamous cell skin cancers (26 28), but the current investigation is the first to document associations for specific NHL subtypes other than CLL/SLL. This study is also the first to show an increased risk for NHL following a diagnosis of actinic keratosis, the precursor of squamous cell skin cancer. Although skin damage from ultraviolet radiation strongly increases risk for skin cancer, ultraviolet radiation actually appears inversely associated with NHL (29). More likely, the association of nonmelanoma skin cancer and NHL is related to immunosuppression, as suggested by associations of HIV and solid organ transplantation with skin cancer (30, 31).

Hematologic conditions associated with increased NHL risk in our study have been described as complications of lymphopro liferative conditions (e.g., hemolytic anemia and cryoglobuline mia) or nonspecific manifestations of chronic illness (32 35). It is unlikely that these conditions were caused by undiagnosed NHL (i.e., reverse causality), because we did not consider Medicare claims within 1 year before NHL diagnosis, and remarkably, almost all of the associations persisted in sensitivity analyses excluding claims within 3 years of NHL. Instead, these associa tions may again reflect the presence of chronic immune distur bances that contribute to the development of NHL over a pro longed period.

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Table 4. Replication of selected associations in additional populations

Medical condition	Population	Number of people with medical condition in sample	Relative risk (95% CI)[a]				
			CLL/SLL	DLBCL	FL	MZL	TCL
Hypertension							
	MedWAS		0.83 (0.80 0.86)	0.93 (0.89 0.96)	0.87 (0.83 0.92)	0.80 (0.74 0.86)	0.85 (0.77 0.94)
	NIH AARP	129,350	0.96 (0.82 1.12)	1.01 (0.85 1.21)	1.02 (0.83 1.26)	0.63 (0.45 0.88)	0.77 (0.53 1.13)
	PLCO	50,400	1.18 (0.99 1.40)	1.02 (0.82 1.27)	0.87 (0.66 1.16)	[b]	1.08 (0.65 1.80)
Diabetes mellitus							
	MedWAS		0.96 (0.92 1.00)	1.09 (1.05 1.13)	0.89 (0.84 0.94)	0.77 (0.71 0.83)	0.93 (0.84 1.04)
	NIH AARP	50,748	1.10 (0.91 1.33)	1.17 (0.95 1.45)	0.94 (0.72 1.24)	0.73 (0.45 1.19)	1.85 (1.25 2.73)
	PLCO	11,364	1.28 (0.95 1.73)	1.07 (0.72 1.60)	1.11 (0.67 1.82)	[b]	1.49 (0.68 3.28)
Stroke							
	MedWAS		0.77 (0.74 0.81)	0.86 (0.83 0.91)	0.82 (0.76 0.88)	0.74 (0.67 0.81)	0.79 (0.69 0.90)
	NIH AARP	11,927	0.98 (0.67 1.44)	1.20 (0.80 1.80)	1.04 (0.61 1.76)	0.54 (0.17 1.68)	0.97 (0.36 2.62)
	PLCO	2421	1.30 (0.78 2.17)	1.62 (0.91 2.88)	1.47 (0.69 3.12)	[b]	2.14 (0.67 6.83)
Chronic bronchitis							
	MedWAS		0.82 (0.77 0.89)	0.78 (0.73 0.84)	0.79 (0.71 0.88)	0.95 (0.83 1.08)	0.73 (0.60 0.89)
	PLCO	7071	0.42 (0.22 0.78)	0.72 (0.39 1.31)	1.31 (0.75 2.29)	[b]	1.10 (0.35 3.52)

Abbreviation: FL, follicular lymphoma.

[a]Relative risks from the MedWAS study are highlighted in gray if they were identified in the SELECT3 medical conditions in the MedWAS analyses (see Table 2). Relative risks are underlined if they are statistically significant ($P < 0.05$). Relative risks were calculated in the MedWAS analyses as ORs and in the NIH AARP and PLCO analyses as HRs.

[b]There were no cases of marginal zone lymphoma in the PLCO cohort.

We found strong associations between a large number of dermatologic conditions and risk of TCL, some of which have been observed previously (6, 36). TCLs can have an indolent presentation (37), and 48% of TCLs in our study were cutaneous lymphomas (mycosis fungoides and less common variants). Diagnostic confusion between skin conditions and TCL could be an explanation, but the associations persisted when we excluded diagnoses within 3 years of NHL. Alternatively, these associations may reflect a shared predisposition to skin diseases and TCL, immune effects of chronic skin diseases, or effects of treatment of the skin diseases (38).

The association of gastric ulcer with MZL may be explained by diagnostic confusion between gastric ulcers and gastric MZL, or by the etiologic contribution of *Helicobacter pylori* to both conditions (39, 40). There was also a positive association of diabetes mellitus with DLBCL. This finding showed some evidence, although inconclusive, for replication in our analyses in NIH AARP and PLCO. Diabetes mellitus has previously been associated with increased risk of NHL overall (41, 42).

Some inverse associations with NHL risk could have biologic explanations. Decreased risk of CLL/SLL following a diagnosis of hyperlipidemia is intriguing, and prior studies have noted a protective effect of statins (a widely used class of lipid lowering medications) for NHL overall and leukemia (43). Decreased CLL/SLL risk associated with alcoholism may reflect protective effects of ethanol consumption (3).

Given our lack of data on lifestyle factors, it is possible that some associations could be due to confounding, for example, by smoking, drinking, or occupation (3). Other artifacts could underlie inverse associations. It is likely that clinicians limited the medical work up of some frail and debilitated elderly adults among the Medicare population, which would have led to under ascertainment of NHL. This bias could explain the inverse associations with a broad range of neurologic and psychiatric conditions and conditions associated with advanced illness or nursing home care (e.g., decubitus ulcer, hip fracture). Indeed, we did not observe decreased NHL risk when, in our replication analyses, we assessed people with a history of a stroke in the NIH AARP and PLCO studies. Participants in these cohort studies were younger and would have been healthier than unselected Medicare beneficiaries, and so NHLs arising in these individuals would have been less vulnerable to under diagnosis.

The current study is the first implementation of a new method, "MedWAS," which we used to comprehensively evaluate a very large number of medical conditions as NHL risk factors. Demonstration of multiple known associations with NHL supports the validity of this approach. MedWAS incorporates the same agnostic, wide based approach used in GWAS studies to survey thousands of DNA variations (44). MedWAS could also be applied to other sources of administrative data and electronic health records. Recent "big data" analyses of large administrative databases by others have focused on characterizing the network properties of related medical conditions (45) or, with respect to cancer, selecting optimum patient treatments and predicting outcomes (46).

Several limitations of our study should be noted. First, it was restricted to the U.S. elderly, and our results may not generalize to other populations. Although Medicare covers essentially all U.S. adults over the age of 65 years, our requirements that subjects were not in an HMO and had at least one documented claim led to some exclusions. Furthermore, we assessed Medicare data beginning at the age of 65 years, so we were unable to evaluate medical conditions that did not generate claims at older ages. Second, we are unaware of a systematic method for using ICD 9 codes to classify unique medical conditions at a consistent and informative level of detail. We therefore found it necessary to review individual codes to identify biologically relevant conditions and to eliminate invalid or irrelevant codes, which likely introduced some subjectivity. Medicare claims can be inaccurate, but we sought to increase the positive predictive value by requiring one inpatient or two physician/outpatient claims at least 30 days apart (16). Also, it is not possible to assess duration or severity of medical conditions using Medicare claims, and as our study was exploratory, we did not

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Case 3:16-md-02741-VC Document 654-2 Filed 10/28/17 Page 8 of 138

attempt to examine associations with treatments for these conditions. Third, because we made thousands of comparisons, some associations could have been due to chance. We sought to minimize this issue by requiring strict statistical significance in the initial screening of the ICD 9 codes and by attempting to independently replicate some findings. Unfortunately, it was challenging to find appropriate data sources for replication, and due to the small number of outcomes and modest size of the associations, the replication analyses were inconclusive.

In conclusion, our study comprehensively assessed a very large number of medical conditions and thereby identified a subset associated with increased or decreased NHL risk. Specific associations varied according to NHL subtype. Many risk factors were related to immune disturbances and chronic infections, as expected, but some (such as the associations with nonmelanoma skin cancer, skin conditions, and diabetes) point to new avenues for research. It will be important to replicate some findings in additional populations, and to uncover biologic mechanisms underpinning the best supported and strongest associations. We believe that this MedWAS approach can be useful in epidemiologic research aimed at understanding the etiology of cancer and other complex diseases.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Disclaimer

The statements contained herein are solely those of the authors and do not represent or imply concurrence or endorsement by the NCI.

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Case 3:16-md-02741-VC Document 654-2 Filed 10/28/17 Page 9 of 138

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Supplemental Table 1. Power calculations for replication analyses

Cohort for replication	Medical condition	NHL subtype	OR from MedWAS	Prevalence of medical condition in cohort	Total number of cases of NHL outcomes in cohort	Power to replicate OR
PLCO	Hypertension	CLL/SLL	0.83	0.342	546	0.45
PLCO	Diabetes	DLBCL	1.09	0.077	362	0.07
PLCO	Stroke	CLL/SLL	0.77	0.024	545	0.08
PLCO	Stroke	DLBCL	0.86	0.024	362	0.04
PLCO	Stroke	FL	0.82	0.024	231	0.04
PLCO	Stroke	MZL	0.74	0.024	65	0.03
PLCO	Chronic bronchitis	DLBCL	0.78	0.048	362	0.04
NIH-AARP	Hypertension	CLL/SLL	0.83	0.435	649	0.52
NIH-AARP	Diabetes	DLBCL	1.09	0.092	962	0.14
NIH-AARP	Stroke	CLL/SLL	0.77	0.022	1182	0.15
NIH-AARP	Stroke	DLBCL	0.86	0.022	962	0.08
NIH-AARP	Stroke	FL	0.82	0.022	679	0.11
NIH-AARP	Stroke	MZL	0.74	0.022	265	0.19

Notes on replication cohorts and statistical analyses

The National Institutes of Health-AARP Diet and Health Study (NIH-AARP) cohort includes 566,407 people enrolled in the US during 1995-1996 (ages 50-71 years at enrollment). We utilized data from the NIH-AARP study baseline questionnaire regarding past history of diabetes mellitus (N=547,008 participants evaluable) and stroke (N=550,294); NIH-AARP data on hypertension were obtained from a follow-up questionnaire and were available for fewer participants (N=297,260). Associations of these medical conditions with NHL outcomes were calculated as hazard ratios using Cox proportional hazards models adjusted for sex and race, with age as the timescale.

The Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial (PLCO) cohort comprises 154,897 people enrolled in a US screening trial during 1993-2001 (ages 55-74 years at enrollment). Data on past history of hypertension, diabetes mellitus, stroke, and chronic bronchitis were ascertained from the baseline PLCO questionnaire (N=148,373 evaluable participants). Associations with NHL outcomes were assessed using Cox models adjusted for sex, race, and year of enrollment, with age as the timescale.

Case 3:16-md-02741-VC Document 654-2 Filed 10/28/17 Page 10 of 138

Case 3:16-md-02741-VC Document 654-2 Filed 10/28/17 Page 11 of 138

Supplemental Table 2. SELECT1 conditions associated with subtypes of NHL

NHL subtype	ICD-9 code	Label	Exposed Cases	Percent of Cases Exposed	Adjusted odds ratio*	Lower 95%CI	Upper 95%CI	Cochran-Mantel Haenszel p-value
CLL/SLL	053.9	Herpes zoster without mention of complication	251	1.38	1.55	1.35	1.77	7.07E-13
CLL/SLL	173.2	Other malignant neoplasm of skin of ear and external auditory canal	192	1.05	1.58	1.36	1.85	3.47E-11
CLL/SLL	173.3	Other malignant neoplasm of skin of other and unspecified parts of face	965	5.29	1.24	1.16	1.33	2.18E-13
CLL/SLL	173.4	Other malignant neoplasm of skin of scalp and skin of neck	210	1.15	1.40	1.21	1.62	1.56E-07
CLL/SLL	238.2	Neoplasm of uncertain behavior of skin	1140	6.25	1.16	1.08	1.23	3.80E-09
CLL/SLL	272.4	Other and unspecified hyperlipidaemia	5493	30.12	0.86	0.83	0.90	1.21E-06
CLL/SLL	273.1	Monoclonal paraproteinaemia	76	0.42	2.00	1.56	2.57	5.54E-10
CLL/SLL	279.0	Deficiency of humoral immunity	26	0.14	3.86	2.37	6.30	7.97E-12
CLL/SLL	282.9	Hereditary hemolytic anemia, unspecified	22	0.12	3.56	1.97	6.42	1.08E-08
CLL/SLL	283.0	Autoimmune hemolytic anemias	40	0.22	7.22	4.41	11.81	5.82E-32
CLL/SLL	287.3	Primary thrombocytopenia	77	0.42	2.11	1.64	2.72	1.67E-11
CLL/SLL	287.5	Thrombocytopenia, unspecified	400	2.19	1.72	1.55	1.92	3.84E-29
CLL/SLL	290.0	Senile dementia, uncomplicated	219	1.20	0.54	0.47	0.62	2.38E-16
CLL/SLL	290.2	Senile dementia with delusional or depressive features	56	0.31	0.48	0.36	0.62	5.73E-07
CLL/SLL	290.4	Arteriosclerotic dementia	91	0.50	0.54	0.44	0.67	4.34E-07
CLL/SLL	294.1	Dementia in conditions classified elsewhere (Alzheimer's)	165	0.90	0.57	0.49	0.67	2.37E-10
CLL/SLL	294.8	Other persistent mental disorders due to conditions classified elsewhere (dementia NOS)	311	1.71	0.57	0.51	0.64	2.73E-18
CLL/SLL	298.9	Unspecified psychosis	210	1.15	0.64	0.56	0.74	1.37E-07
CLL/SLL	331.0	Alzheimer's disease	293	1.61	0.56	0.49	0.63	5.27E-19
CLL/SLL	401.1	Essential hypertension, benign	6347	34.80	0.85	0.82	0.88	2.05E-07
CLL/SLL	401.9	Essential hypertension, unspecified	8387	45.99	0.84	0.81	0.87	1.39E-06
CLL/SLL	434.9	Cerebral artery occlusion, unspecified	295	1.62	0.69	0.61	0.77	6.20E-07

CLL/SLL	435.9	Unspecified transient cerebral ischemia	524	2.87	0.70	0.64	0.76	2.82E-10
CLL/SLL	436	Acute but ill-defined cerebrovascular disease	757	4.15	0.78	0.72	0.84	5.27E-06
CLL/SLL	441.4	Abdominal aneurysm without mention of rupture	196	1.07	0.67	0.58	0.77	4.54E-06
CLL/SLL	461.9	Acute sinusitis, unspecified	769	4.22	1.14	1.06	1.24	1.14E-06
CLL/SLL	466.0	Acute bronchitis	2075	11.38	1.06	1.01	1.12	4.80E-07
CLL/SLL	702.0	Actinic keratosis	3012	16.52	1.12	1.07	1.17	7.39E-13
CLL/SLL	713.6	Arthropathy associated with hypersensitivity reaction	<11	--	--	--	--	2.84E-06
CLL/SLL	742.5	Other specified anomalies of spinal cord	27	0.15	3.21	2.01	5.13	2.70E-09
DLBCL	042	Human immunodeficiency virus (HIV) infection	17	0.09	3.76	1.54	9.22	1.90E-07
DLBCL	070.5	Other specified viral hepatitis without mention of hepatic coma	77	0.40	1.74	1.35	2.24	1.07E-06
DLBCL	135	Sarcoidosis	41	0.21	2.13	1.49	3.04	2.03E-06
DLBCL	173.2	Other malignant neoplasm of skin of ear and external auditory canal	163	0.85	1.52	1.29	1.80	1.39E-07
DLBCL	173.3	Other malignant neoplasm of skin of other and unspecified parts of face	958	5.02	1.26	1.17	1.35	5.71E-13
DLBCL	173.6	Other malignant neoplasm of skin of upper limb, including shoulder	226	1.18	1.33	1.16	1.54	7.69E-06
DLBCL	238.2	Neoplasm of uncertain behavior of skin	1108	5.81	1.12	1.05	1.20	7.53E-06
DLBCL	250.0	Diabetes mellitus without mention of complication	4500	23.59	1.09	1.05	1.13	3.17E-11
DLBCL	273.1	Monoclonal paraproteinaemia	69	0.36	1.80	1.39	2.34	8.59E-07
DLBCL	273.2	Other paraproteinaemias	<11	--	6.26	1.34	29.30	7.06E-06
DLBCL	283.0	Autoimmune hemolytic anemias	25	0.13	4.35	2.46	7.69	2.33E-12
DLBCL	287.3	Primary thrombocytopenia	74	0.39	1.97	1.52	2.54	3.59E-09
DLBCL	287.5	Thrombocytopenia, unspecified	337	1.77	1.44	1.28	1.62	4.24E-12
DLBCL	290.0	Senile dementia, uncomplicated	157	0.82	0.36	0.31	0.42	8.37E-35
DLBCL	290.1	Presenile dementia	41	0.21	0.32	0.23	0.44	2.76E-13
DLBCL	290.2	Senile dementia with delusional or depressive features	39	0.20	0.32	0.23	0.44	2.26E-12
DLBCL	290.4	Arteriosclerotic dementia	61	0.32	0.35	0.27	0.45	1.79E-15

Case 3:16-md-02741-VC Document 654-2 Filed 10/28/17 Page 12 of 138

Case 3:16-md-02741-VC Document 654-2 Filed 10/28/17 Page 13 of 138

DLBCL	294.1	Dementia in conditions classified elsewhere	112	0.59	0.37	0.30	0.45	1.93E-24
DLBCL	294.8	Other persistent mental disorders due to conditions classified elsewhere (dementia NOS)	261	1.37	0.45	0.40	0.51	2.66E-33
DLBCL	298.9	Unspecified psychosis	160	0.84	0.47	0.40	0.55	2.98E-18
DLBCL	303.9	Other and unspecified alcohol dependence	73	0.38	0.54	0.43	0.69	1.49E-06
DLBCL	310.9	Unspecified nonpsychotic mental disorder following organic brain damage	21	0.11	0.23	0.15	0.36	2.76E-12
DLBCL	311	Depressive disorder, not elsewhere classified	946	4.96	0.75	0.70	0.80	5.20E-11
DLBCL	331.0	Alzheimer's disease	227	1.19	0.41	0.36	0.47	2.20E-38
DLBCL	332.0	Parkinson's disease	239	1.25	0.67	0.59	0.77	1.25E-07
DLBCL	363.2	Other and unspecified forms of chorioretinitis and retinochoroiditis	23	0.12	3.17	1.86	5.40	1.59E-07
DLBCL	385.1	Adhesive middle ear disease	<11	--	6.07	0.01	>1000	8.97E-06
DLBCL	434.9	Cerebral artery occlusion, unspecified	323	1.69	0.73	0.65	0.82	9.75E-06
DLBCL	436	Acute but ill-defined cerebrovascular disease	775	4.06	0.77	0.72	0.83	2.82E-07
DLBCL	437.1	Other generalised ischemic cerebrovascular disease	59	0.31	0.51	0.39	0.67	3.02E-06
DLBCL	438	Late effects of cerebrovascular disease	124	0.65	0.61	0.51	0.73	4.91E-06
DLBCL	491.2	Obstructive chronic bronchitis	706	3.70	0.75	0.69	0.81	8.95E-09
DLBCL	527.2	Sialoadenitis	63	0.33	1.84	1.40	2.43	1.53E-06
DLBCL	599.0	Urinary tract infection, site not specified	3160	16.56	0.83	0.79	0.87	9.08E-09
DLBCL	702.0	Actinic keratosis	3015	15.80	1.14	1.09	1.19	5.78E-13
DLBCL	707.0	Decubitus ulcer	153	0.80	0.57	0.48	0.67	1.47E-09
DLBCL	710.0	Systemic lupus erythematosus	95	0.50	1.73	1.38	2.17	4.06E-08
DLBCL	710.2	Sicca syndrome	111	0.58	2.39	1.93	2.95	7.44E-19
DLBCL	714.0	Rheumatoid arthritis	767	4.02	1.43	1.32	1.55	1.49E-24
DLBCL	806.9	Fracture of vertebral column with spinal cord injury, unspecified open	<11	--	--	--	--	2.73E-06
DLBCL	820.8	Fracture of unspecified part of neck of femur (hip), closed	218	1.14	0.62	0.54	0.71	5.40E-10
DLBCL	850.3	Concussion, with prolonged loss of consciousness and return to pre-existing conscious level	<11	--	--	--	--	2.54E-10

DLBCL	996.8	Complications of transplanted organ	40	0.21	3.77	2.50	5.69	1.92E-15
FL	013	Tuberculosis of meninges and central nervous system	<11	--	--	--	--	1.10E-06
FL	173.3	Other malignant neoplasm of skin of other and unspecified parts of face	404	4.55	1.23	1.11	1.37	5.27E-06
FL	173.9	Other malignant neoplasm of skin, site unspecified	150	1.69	1.47	1.25	1.74	8.51E-07
FL	238.2	Neoplasm of uncertain behavior of skin	570	6.42	1.30	1.18	1.42	8.48E-11
FL	273.2	Other paraproteinaemias	<11	--	10.50	1.85	59.48	8.37E-09
FL	283.0	Autoimmune hemolytic anemias	<11	--	3.90	1.76	8.62	9.91E-06
FL	290.0	Senile dementia, uncomplicated	62	0.70	0.39	0.30	0.50	3.26E-13
FL	290.1	Presenile dementia	15	0.17	0.30	0.18	0.51	2.72E-06
FL	290.2	Senile dementia with delusional or depressive features	13	0.15	0.28	0.16	0.48	2.11E-06
FL	290.4	Arteriosclerotic dementia	21	0.24	0.32	0.21	0.49	7.30E-08
FL	294.1	Dementia in conditions classified elsewhere	32	0.36	0.27	0.19	0.39	6.27E-14
FL	294.8	Other persistent mental disorders due to conditions classified elsewhere (dementia NOS)	83	0.93	0.38	0.30	0.47	1.68E-18
FL	298.9	Unspecified psychosis	50	0.56	0.38	0.29	0.50	1.30E-11
FL	311	Depressive disorder, not elsewhere classified	400	4.50	0.70	0.63	0.78	1.64E-08
FL	331.0	Alzheimer's disease	71	0.80	0.33	0.26	0.42	1.24E-20
FL	348	Other conditions of brain	<11	--	--	--	--	2.28E-06
FL	375.6	Other changes of lachrymal passages	<11	--	--	--	--	3.41E-12
FL	428.0	Congestive heart failure, unspecified	829	9.33	0.78	0.73	0.85	4.61E-06
FL	436	Acute but ill-defined cerebrovascular disease	272	3.06	0.66	0.58	0.75	5.77E-09
FL	456.3	Sublingual varices	<11	--	--	--	--	4.18E-11
FL	702.0	Actinic keratosis	1426	16.06	1.20	1.13	1.28	1.14E-12
FL	702.1	Seborrheic keratosis	775	8.73	1.18	1.09	1.27	3.06E-07
FL	799.0	Asphyxia	55	0.62	0.53	0.40	0.69	1.03E-05
FL	820.8	Fracture of unspecified part of neck of femur (hip), closed	69	0.78	0.50	0.39	0.64	5.49E-08
FL	940.3	Acid chemical burn of cornea and conjunctival sac	<11	--	--	--	--	1.27E-06

Case 3:16-md-02741-VC Document 654-2 Filed 10/28/17 Page 14 of 138

Case 3:16-md-02741-VC Document 654-2 Filed 10/28/17 Page 15 of 138

FL	951.8	Injury to other specified cranial nerves	<11	--	--	--	--	2.08E-08
FL	987.0	Toxic effect of liquefied petroleum gases	<11	--	--	--	--	2.33E-08
MZL	041	Bacterial infection in conditions classified elsewhere and of unspecified site	<11	--	--	--	--	5.76E-15
MZL	118	Opportunistic mycoses	<11	--	32.24	2.02	515.04	5.93E-06
MZL	125.6	Other specified filariasis	<11	--	41.70	2.62	662.97	6.63E-06
MZL	238.2	Neoplasm of uncertain behavior of skin	339	7.90	1.41	1.26	1.59	4.15E-12
MZL	257.9	Unspecified testicular dysfunction	<11	--	8.40	2.82	25.04	3.15E-09
MZL	259	Other endocrine disorders	<11	--	--	--	--	2.68E-08
MZL	273.1	Monoclonal paraproteinaemia	34	0.79	3.47	2.43	4.96	1.23E-15
MZL	273.2	Other paraproteinaemias	<11	--	9.72	1.21	77.71	8.16E-06
MZL	282.8	Other specified hereditary hemolytic anemias	<11	--	--	--	--	1.27E-16
MZL	282.9	Hereditary hemolytic anemia, unspecified	<11	--	4.53	1.76	11.69	9.68E-07
MZL	283.0	Autoimmune hemolytic anemias	<11	--	7.21	3.18	16.34	2.01E-12
MZL	283.9	Acquired hemolytic anemia, unspecified	<11	--	3.86	1.87	7.96	1.56E-07
MZL	284.1	Pancytopenia	<11	--	8.48	2.88	24.95	1.76E-09
MZL	284.8	Other specified aplastic anemias	59	1.38	3.45	2.63	4.53	2.67E-27
MZL	285.9	Anemia, unspecified	914	21.31	1.12	1.04	1.22	1.09E-08
MZL	287	Purpura and other hemorrhagic conditions	<11	--	--	--	--	8.85E-12
MZL	287.3	Primary thrombocytopenia	28	0.65	2.95	1.99	4.38	1.89E-10
MZL	287.5	Thrombocytopenia, unspecified	123	2.87	2.15	1.78	2.59	2.61E-21
MZL	288.0	Neutropenia	66	1.54	2.46	1.91	3.17	1.31E-16
MZL	290.0	Senile dementia, uncomplicated	39	0.91	0.39	0.28	0.54	7.73E-08
MZL	294.1	Dementia in conditions classified elsewhere	27	0.63	0.34	0.23	0.50	1.21E-07
MZL	294.8	Other persistent mental disorders due to conditions classified elsewhere (dementia NOS)	63	1.47	0.42	0.33	0.54	5.86E-10
MZL	298.9	Unspecified psychosis	29	0.68	0.37	0.26	0.53	1.52E-06
MZL	302.5	Trans-sexualism	<11	--	--	--	--	1.28E-12
MZL	331.0	Alzheimer's disease	55	1.28	0.40	0.31	0.53	3.43E-10
MZL	392	Rheumatic chorea	<11	--	48.66	3.06	774.40	3.78E-06
MZL	436	Acute but ill-defined cerebrovascular disease	145	3.38	0.59	0.50	0.70	4.16E-06

MZL	517.8	Lung involvement in other diseases classified elsewhere	11	0.26	5.09	2.56	10.12	1.20E-09
MZL	527.2	Sialoadenitis	48	1.12	5.62	4.12	7.66	4.51E-40
MZL	527.3	Abscess of salivary gland	<11	--	14.11	0.00	>1000	2.16E-07
MZL	529.4	Atrophy of tongue papillae	<11	--	--	--	--	2.16E-12
MZL	531.4	Gastric ucler, chronic or unspecified with hemorrhage	41	0.96	1.97	1.43	2.71	1.24E-06
MZL	600.0	Hypertrophy (benign) of prostate	462	10.77	1.24	1.11	1.39	1.34E-06
MZL	614.7	Other chronic pelvic peritonitis, female	<11	--	--	--	--	8.74E-11
MZL	638.5	Failed attempted abortion, complicated by shock	<11	--	--	--	--	2.10E-09
MZL	702.0	Actinic keratosis	746	17.39	1.17	1.08	1.27	1.90E-06
MZL	702.1	Seborrheic keratosis	447	10.42	1.24	1.12	1.37	1.90E-07
MZL	710.0	Systemic lupus erythematosus	39	0.91	2.55	1.83	3.57	4.92E-11
MZL	710.2	Sicca syndrome	56	1.31	4.53	3.41	6.03	8.26E-36
MZL	790.1	Elevated sedimentation rate	32	0.75	2.00	1.39	2.87	4.41E-06
MZL	837.1	Dislocation of ankle, open	<11	--	--	--	--	6.54E-08
MZL	890	Open wound of hip and thigh	<11	--	--	--	--	6.24E-10
MZL	902.8	Injury to blood vessels of abdomen and pelvis, other specified blood vessels	<11	--	--	--	--	3.52E-12
MZL	913.7	and wrist, superficial foreign body	<11	--	--	--	--	3.34E-08
MZL	955.4	Injury to musculocutaneous nerve	<11	--	--	--	--	4.03E-09
MZL	958.1	Fat embolism	<11	--	12.24	0.00	>1000	4.12E-06
MZL	958.6	Volkmann's ischemic contracture	<11	--	--	--	--	1.24E-10
MZL	960.9	Poisoning by unspecified antibiotic	<11	--	--	--	--	5.55E-13
MZL	976.0	Poisoning by local anti-infectives and anti-inflammatory drugs	<11	--	--	--	--	8.04E-10
MZL	992.2	Heat cramps	<11	--	--	--	--	7.75E-08
TCL	014	Tuberculosis of intestines, peritoneum, and mesenteric glands	<11	--	27.85	0.00	>1000	1.62E-06
TCL	061	Dengue	<11	--	--	--	--	1.17E-08
TCL	098.1	Acute gonococcal infection of upper genitourinary tract	<11	--	--	--	--	2.51E-13
TCL	098.8	Gonococcal infection of other specified sites	<11	--	96.53	6.03	>1000	5.53E-14
TCL	104.9	Spirochaetal infection, unspecified	<11	--	--	--	--	5.71E-07
TCL	110.5	Dermatophytosis of the body	15	0.68	2.99	1.77	5.06	3.44E-06
TCL	110.9	Dermatophytosis of unspecified site	15	0.68	3.36	1.98	5.70	1.02E-07

Case 3:16-md-02741-VC Document 654-2 Filed 10/28/17 Page 16 of 138

Case 3:16-md-02741-VC Document 654-2 Filed 10/28/17 Page 17 of 138

TCL	111	Dermatomycosis, other and unspecified	<11	--	--	--	--	5.05E-16
TCL	173.2	Other malignant neoplasm of skin of ear and external auditory canal	31	1.40	2.38	1.65	3.43	4.94E-08
TCL	173.3	Other malignant neoplasm of skin of other and unspecified parts of face	128	5.80	1.55	1.29	1.87	1.38E-08
TCL	173.6	Other malignant neoplasm of skin of upper limb, including shoulder	39	1.77	2.07	1.50	2.86	3.34E-07
TCL	238.2	Neoplasm of uncertain behavior of skin	181	8.20	1.71	1.46	2.01	1.37E-15
TCL	257.1	Postablative testicular hypofunction	<11	--	17.45	1.04	291.48	2.07E-12
TCL	279.0	Deficiency of humoral immunity	<11	--	5.69	2.12	15.26	1.19E-06
TCL	283.0	Autoimmune hemolytic anemias	<11	--	7.34	2.42	22.23	7.53E-09
TCL	287.3	Primary thrombocytopenia	16	0.73	3.39	2.02	5.69	1.13E-08
TCL	320	Bacterial meningitis	<11	--	--	--	--	1.91E-28
TCL	327.3	Circadian rhythm sleep disorder	<11	--	--	--	--	3.34E-13
TCL	359.2	Myotonic disorders	<11	--	35.67	3.61	351.91	6.43E-06
TCL	385.1	Adhesive middle ear disease	<11	--	13.75	0.00	>1000	5.38E-06
TCL	415	Acute pulmonary heart disease	<11	--	37.19	6.60	209.75	5.03E-12
TCL	453	Other venous embolism and thrombosis	<11	--	--	--	--	1.43E-10
TCL	471.1	Polypoid sinus degeneration	<11	--	11.45	2.30	56.88	1.50E-07
TCL	528.7	Other disturbances of oral epithelium, including tongue	<11	--	13.91	1.93	100.16	7.79E-10
TCL	579.0	Celiac disease	11	0.50	8.28	4.32	15.88	6.16E-17
TCL	581.2	Nephrotic syndrome, with lesion of membranoproliferative glomerulonephritis	<11	--	--	--	--	7.37E-06
TCL	611.2	Fissure of nipple	<11	--	53.04	4.80	586.57	7.09E-09
TCL	691.8	Other atopic dermatitis and related conditions	76	3.44	4.16	3.27	5.28	6.18E-44
TCL	692.3	Contact dermatitis and other eczema, due to drugs and medicines in contact with skin	<11	--	4.12	1.97	8.65	1.52E-06
TCL	692.8	Contact dermatitis and other eczema, due to other specified agents	19	0.86	3.50	2.19	5.59	1.19E-10
TCL	692.9	Contact dermatitis and other eczema, unspecified cause	363	16.45	3.06	2.71	3.45	5.82E-103

TCL	693.0	Dermatitis due to substances taken internally, due to drugs and medicines	27	1.22	4.20	2.83	6.25	2.91E-19
TCL	694.5	Pemphigoid	<11	--	5.85	2.78	12.30	1.13E-09
TCL	694.8	Other specified bullous dermatoses	<11	--	29.85	0.00	>1000	6.94E-13
TCL	695.2	Erythema nodosum	<11	--	9.07	1.61	50.95	1.12E-06
TCL	695.4	Discoid lupus erythematosus	<11	--	4.04	2.01	8.13	1.49E-06
TCL	696.1	Other psoriasis	85	3.85	3.20	2.56	4.01	5.37E-32
TCL	696.2	Parapsoriasis	22	1.00	46.13	25.14	84.63	2.02E-138
TCL	696.3	Pityriasis rosea	<11	--	11.05	2.78	43.93	1.02E-10
TCL	702.0	Actinic keratosis	366	16.58	1.25	1.11	1.41	2.10E-07
TCL	704.8	Other specified diseases of hair and hair follicles	22	1.00	2.66	1.72	4.10	1.44E-07
TCL	706.8	Other specified diseases of sebaceous glands	45	2.04	2.19	1.62	2.96	4.95E-10
TCL	708.1	Idiopathic urticaria	<11	--	6.23	1.87	20.78	4.72E-06
TCL	708.8	Other specified urticaria	<11	--	8.00	3.19	20.08	5.38E-10
TCL	711.6	Arthropathy associated with mycoses	<11	--	26.52	2.74	257.00	2.08E-06
TCL	736.5	Genu recurvatum (acquired)	<11	--	61.01	5.51	675.92	2.33E-08
TCL	890	Open wound of hip and thigh	<11	--	--	--	--	2.47E-17
TCL	897.6	Traumatic amputation of leg(s), without mention of complication	<11	--	8.28	0.31	221.71	6.50E-06
TCL	905.7	Late effect of sprain and strain without mention of tendon injury	<11	--	8.26	1.96	34.76	9.54E-06
TCL	913.7	Superficial injury of elbow, forearm, and wrist, superficial foreign body (splinter) without major open wound, infected	<11	--	--	--	--	2.73E-21
TCL	951.0	Injury to oculomotor nerve	<11	--	--	--	--	5.51E-09
TCL	955.1	Injury to median nerve	<11	--	9.65	0.38	247.88	4.61E-06

Supplemental Table 2 notes

In accordance with the SEER-Medicare data use agreement, counts less than 11 (and the corresponding percentages) are suppressed.

* Odds ratios are adjusted for sex, age and calendar year of case/control selection, race, and number of provider claims per year. Results are not shown for models that failed to converge.

Case 3:16-md-02741-VC Document 654-2 Filed 10/28/17 Page 18 of 138

Case 3:16-md-02741-VC Document 654-2 Filed 10/28/17 Page 19 of 138

Supplemental Table 3. ICD9 codes and counts of the number of NHL cases with each SELECT3 condition

Condition	ICD9 codes	CLL/SLL N (%)	DLBCL N (%)	FL N (%)	MZL N (%)	TCL N (%)
IMMUNODEFICIENCY RELATED CONDITIONS						
HIV	042-044, V08	-*	20 (0.10)	-*	-*	0
Deficiency of humoral immunity	279.0	26 (0.14)	-*	-*	-*	-*
Solid organ transplantation	996.80-996.84, 996.86-996.89, V42.0, V42.1, V42.6, , V42.7, V42.83, V42.84, V42.9	15 (0.08)	73 (0.38)	-*	-*	-*
INFECTIONS						
Herpes zoster	053	350 (1.92)	326 (1.71)	117 (1.32)	91 (2.12)	35 (1.59)
HCV	070.51, 070.54, 070.7	25 (0.14)	79 (0.41)	13 (0.15)	16 (0.37)	-*
Acute sinusitis	461	1112 (6.10)	1064 (5.58)	569 (6.41)	305 (7.11)	121 (5.48)
Acute bronchitis	466.0	2075 (11.38)	2095 (10.98)	902 (10.16)	545 (12.71)	250 (11.33)
Chronic bronchitis	491	880 (4.83)	895 (4.69)	390 (4.39)	268 (6.25)	104 (4.71)
Urinary tract infection	599.0	2986 (16.37)	3160 (16.56)	1355 (15.26)	762 (17.77)	333 (15.09)
AUTOIMMUNE AND INFLAMMATORY CONDITIONS						
Rheumatoid arthritis	714.0	441 (2.42)	767 (4.02)	291 (3.28)	164 (3.82)	85 (3.85)
Systemic lupus erythematosus	710.0	40 (0.22)	95 (0.50)	28 (0.32)	39 (0.91)	15 (0.68)
Sjogren syndrome	527.2, 710.2	74 (0.41)	168 (0.88)	58 (0.65)	97 (2.26)	16 (0.72)
Sarcoidosis	135	16 (0.09)	41 (0.21)	-*	13 (0.30)	-*
Celiac disease	579.0	17 (0.09)	17 (0.09)	-*	-*	11 (0.50)
Uveitis	363.2	11 (0.06)	23 (0.12)	-*	-*	0
Elevated sedimentation rate	790.1	57 (0.31)	78 (0.41)	20 (0.23)	32 (0.75)	-*
HEMATOLOGIC CONDITIONS						
Hemolytic anemia	282.8, 282.9, 283.0, 283.9	66 (0.36)	39 (0.20)	14 (0.16)	19 (0.44)	-*
Aplastic anemia	284.1, 284.8, 284.9	112 (0.61)	109 (0.57)	38 (0.43)	65 (1.52)	12 (0.54)
Anemia NOS	285.9	3177 (17.42)	3440 (18.03)	1265 (14.24)	914 (21.31)	395 (17.90)
Thrombocytopenia	287.3, 287.5	429 (2.35)	366 (1.92)	109 (1.23)	135 (3.15)	47 (2.13)
Neutropenia	288.0	127 (0.70)	146 (0.77)	57 (0.64)	66 (1.54)	21 (0.95)
Monoclonal paraproteinaemia	273.1	76 (0.42)	69 (0.36)	24 (0.27)	34 (0.79)	-*
Cryoglobulinemia	273.2	-*	-*	-*	-*	-*
CARDIOVASCULAR DISORDERS						
Benign/unspecified hypertension	401.1, 401.9	10484 (57.49)	11510 (60.33)	5094 (57.36)	2662 (62.07)	1327 (60.13)
Systolic heart failure	428	2338 (12.82)	2450 (12.84)	895 (10.08)	477 (11.12)	257 (11.64)
Abdominal aneurysm	441.4	196 (1.07)	292 (1.53)	101 (1.14)	61 (1.42)	31 (1.40)
ENDOCRINE/METABOLIC CONDITIONS						
Diabetes mellitus	250.0	3878 (21.27)	4500 (23.59)	1752 (19.73)	864 (20.14)	514 (23.29)
Hyperlipidemia NOS	272.4	5493 (30.12)	6259 (32.81)	2921 (32.89)	1507 (35.14)	710 (32.17)
Testicular hypofunction	257.1, 257.2, 257.8, 257.9	108 (0.59)	96 (0.50)	54 (0.61)	40 (0.93)	12 (0.54)

NEUROLOGICAL/PSYCHIATRIC DISORDERS						
Dementia, senile or other causes	290.0, 290.1, 290.2, 290.3, 294.1, 294.8, 331.0	707 (3.88)	550 (2.88)	191 (2.15)	123 (2.87)	64 (2.90)
Arteriosclerotic dementia	290.4	91 (0.50)	61 (0.32)	21 (0.24)	14 (0.33)	-*
Parkinson's disease	332.0	259 (1.42)	239 (1.25)	103 (1.16)	46 (1.07)	26 (1.18)
Stroke, including TIA	433-436, 436, 437.1, 438	2291 (12.56)	2587 (13.56)	1033 (11.63)	557 (12.99)	288 (13.05)
Depression NOS	311	947 (5.19)	950 (4.98)	401 (4.52)	242 (5.64)	119 (5.39)
Psychosis NOS	289.9	210 (1.15)	160 (0.84)	50 (0.56)	29 (0.68)	17 (0.77)
Nonpsychotic mental disorder NOS	310.9	50 (0.27)	21 (0.11)	-*	-*	-*
Alcoholism	303.9, 305.0	178 (0.98)	126 (0.66)	58 (0.65)	40 (0.93)	30 (1.36)
SKIN DISEASES						
Non-melanoma skin cancer	173, 238.2	2425 (13.30)	2421 (12.69)	1114 (12.54)	591 (13.78)	331 (15.00)
Actinic keratosis	702.0	3012 (16.52)	3015 (15.80)	1426 (16.06)	746 (17.39)	366 (16.58)
Atopic dermatitis	691.8	134 (0.73)	184 (0.96)	64 (0.72)	51 (1.19)	76 (3.44)
Contact dermatititis and other eczema	692	1468 (8.05)	1800 (9.43)	742 (8.35)	458 (10.68)	431 (19.53)
Dermatitis due to substances taken internally	693	61 (0.33)	67 (0.35)	23 (0.26)	23 (0.54)	33 (1.50)
Bullous skin diseases	694	24 (0.13)	29 (0.15)	12 (0.14)	-*	-*
Discoid lupus	695.4	-*	36 (0.19)	-*	-*	-*
Psoriasis and similar disorders	696	203 (1.11)	318 (1.67)	117 (1.32)	83 (1.94)	108 (4.89)
Seborrheic keratosis	702.1	1424 (7.81)	1464 (7.67)	775 (8.73)	447 (10.42)	196 (8.88)
Folliculitis and related conditions	704.8	65 (0.36)	69 (0.36)	15 (0.17)	19 (0.44)	22 (1.00)
Asteatosis	706.8	146 (0.80)	174 (0.91)	74 (0.83)	48 (1.12)	45 (2.04)
Urticaria	708	98 (0.54)	126 (0.66)	60 (0.68)	30 (0.70)	35 (1.59)
MISCELLANEOUS CONDITIONS						
Benign prostate hyperplasia	222.2, 600.0, 600.9	2062 (11.31)	1773 (9.29)	828 (9.32)	516 (12.03)	241 (10.92)
Gastric ulcer	531	259 (1.42)	276 (1.45)	112 (1.26)	113 (2.63)	32 (1.45)
Decubitus ulcer	707.0	201 (1.10)	153 (0.80)	60 (0.68)	32 (0.75)	21 (0.95)
Specified anomalies of spinal cord (myelodysplasia)	742.5	27 (0.15)	15 (0.08)	-*	-*	-*
Asphyxia	799.0	207 (1.14)	191 (1.00)	55 (0.62)	37 (0.86)	28 (1.27)
Hip fracture	820.8	243 (1.33)	218 (1.14)	69 (0.78)	59 (1.38)	21 (0.95)

Supplemental Table 3 notes

* Cells with fewer than 11 people are suppressed, in accordance with the SEER-Medicare data use agreement

Case 3:16-md-02741-VC Document 654-2 Filed 10/28/17 Page 20 of 138

Case 3:16-md-02741-VC Document 654-2 Filed 10/28/17 Page 21 of 138

Supplementary Table 4. Sensitivity analysis excluding conditions diagnosed within 3 years of NHL or control selection

	Adjusted odds ratio (95%CI)				
Condition	**CLL/SLL**	**DLBCL**	**FL**	**MZL**	**TCL**
IMMUNODEFICIENCY RELATED CONDITIONS					
HIV	0.65 (0.16-2.69)	2.29 (1.01-5.19)	0	0	0
Deficiency of humoral immunity	2.73 (1.24-6.01)	1.55 (0.60-4.01)	0	2.25 (0.52-9.73)	7.65 (2.33-25.14)
Solid organ transplantation	1.08 (0.58-2.04)	4.08 (2.87-5.80)	0.60 (0.19-1.89)	3.09 (1.55-6.15)	4.64 (2.16-9.94)
INFECTIONS					
Herpes zoster	1.38 (1.18-1.62)	1.30 (1.11-1.52)	0.96 (0.73-1.26)	1.84 (1.42-2.38)	1.27 (0.81-2.01)
HCV	0.72 (0.43-1.22)	2.01 (1.47-2.75)	0.47 (0.19-1.13)	0.75 (0.31-1.82)	1.24 (0.46-3.34)
Acute sinusitis	1.13 (1.03-1.23)	1.01 (0.93-1.10)	1.19 (1.06-1.34)	1.15 (0.98-1.35)	0.89 (0.68-1.15)
Acute bronchitis	1.09 (1.02-1.17)	1.00 (0.94-1.07)	0.95 (0.86-1.05)	1.15 (1.02-1.30)	0.92 (0.77-1.11)
Chronic bronchitis	0.82 (0.75-0.91)	0.83 (0.76-0.91)	0.82 (0.71-0.94)	0.93 (0.78-1.10)	0.76 (0.58-1.00)
Urinary tract infection	0.97 (0.92-1.03)	0.91 (0.86-0.96)	0.86 (0.79-0.93)	0.91 (0.82-1.01)	0.92 (0.79-1.07)
AUTOIMMUNE AND INFLAMMATORY CONDITIONS					
Rheumatoid arthritis	0.95 (0.84-1.08)	1.61 (1.46-1.78)	1.20 (1.02-1.41)	1.19 (0.97-1.46)	1.32 (0.99-1.76)
Systemic lupus erythematosus	0.64 (0.40-1.02)	1.93 (1.48-2.52)	1.07 (0.66-1.75)	2.47 (1.62-3.77)	1.88 (0.93-3.82)
Sjogren syndrome	1.13 (0.83-1.54)	2.08 (1.66-2.59)	1.40 (0.96-2.04)	3.81 (2.81-5.18)	1.33 (0.63-2.81)
Sarcoidosis	0.83 (0.44-1.59)	2.22 (1.48-3.34)	0.33 (0.08-1.33)	1.98 (0.92-4.24)	0.58 (0.08-4.17)
Celiac disease	0.93 (0.40-2.13)	1.45 (0.74-2.82)	1.30 (0.48-3.56)	2.80 (1.13-6.93)	6.80 (2.74-16.88)
Uveitis	1.62 (0.70-3.76)	1.98 (0.93-4.23)	1.70 (0.53-5.51)	1.02 (0.14-7.52)	0
Elevated sedimentation rate	1.06 (0.73-1.55)	1.13 (0.80-1.60)	0.75 (0.40-1.41)	1.41 (0.79-2.52)	1.99 (0.94-4.24)
HEMATOLOGIC CONDITIONS					
Hemolytic anemia	4.46 (2.94-6.76)	2.36 (1.42-3.92)	1.40 (0.56-3.50)	5.15 (2.69-9.86)	6.82 (3.07-15.13)
Aplastic anemia	1.10 (0.79-1.54)	1.36 (1.01-1.82)	1.22 (0.77-1.94)	2.80 (1.90-4.13)	1.27 (0.57-2.86)
Anemia NOS	1.05 (0.99-1.11)	1.02 (0.97-1.08)	0.94 (0.86-1.02)	1.05 (0.95-1.17)	1.01 (0.86-1.17)
Thrombocytopenia	1.73 (1.49-2.01)	1.38 (1.17-1.62)	1.01 (0.77-1.34)	1.70 (1.29-2.25)	1.56 (1.04-2.36)
Neutropenia	1.37 (1.06-1.78)	1.49 (1.18-1.87)	1.50 (1.07-2.12)	2.22 (1.55-3.19)	2.06 (1.21-3.52)
Monoclonal paraproteinaemia	1.87 (1.30-2.68)	1.80 (1.25-2.58)	1.36 (0.74-2.51)	3.38 (2.04-5.58)	0.86 (0.21-3.46)
Cryoglobulinemia	6.07 (0.59-62.08)	8.60 (0.98-75.48)	13.81(1.36-140.1)	7.66 (0.37-159.8)	15.86(0.70-357.0)
CARDIOVASCULAR DISORDERS					
Benign/unspecified hypertension	0.92 (0.89-0.96)	0.97 (0.93-1.00)	0.94 (0.89-0.99)	0.87 (0.81-0.94)	0.98 (0.88-1.09)
Systolic heart failure	0.96 (0.90-1.02)	0.93 (0.87-0.99)	0.82 (0.74-0.91)	0.69 (0.60-0.78)	0.76 (0.63-0.91)
Abdominal aneurysm	0.70 (0.57-0.86)	1.07 (0.91-1.27)	1.01 (0.78-1.31)	0.79 (0.54-1.16)	0.70 (0.40-1.22)
ENDOCRINE/METABOLIC CONDITIONS					
Diabetes mellitus	0.97 (0.92-1.02)	1.11 (1.06-1.16)	0.94 (0.88-1.01)	0.81 (0.74-0.89)	0.94 (0.83-1.06)
Hyperlipidemia NOS	0.94 (0.90-0.98)	1.02 (0.98-1.06)	1.03 (0.97-1.09)	0.97 (0.90-1.05)	0.81 (0.72-0.92)

Testicular hypofunction	1.09 (0.85-1.40)	0.89 (0.67-1.19)	1.20 (0.83-1.74)	1.90 (1.28-2.83)	0.84 (0.40-1.76)
NEUROLOGICAL/PSYCHIATRIC DISORDERS					
Dementia, senile or other causes	0.61 (0.54-0.69)	0.39 (0.34-0.45)	0.38 (0.31-0.48)	0.39 (0.30-0.51)	0.41 (0.27-0.62)
Arteriosclerotic dementia	0.55 (0.40-0.78)	0.31 (0.20-0.47)	0.35 (0.18-0.67)	0.45 (0.22-0.90)	0.12 (0.02-0.86)
Parkinson's disease	0.83 (0.71-0.99)	0.65 (0.55-0.78)	0.65 (0.49-0.86)	0.58 (0.39-0.85)	0.57 (0.33-0.99)
Stroke, incl. includes TIA	0.86 (0.81-0.92)	0.88 (0.83-0.93)	0.81 (0.74-0.89)	0.79 (0.70-0.89)	0.78 (0.66-0.93)
Depression NOS	0.92 (0.84-1.01)	0.78 (0.71-0.86)	0.78 (0.67-0.89)	0.79 (0.66-0.95)	1.02 (0.80-1.31)
Psychosis NOS	0.75 (0.62-0.92)	0.49 (0.38-0.62)	0.39 (0.25-0.59)	0.36 (0.21-0.63)	0.17 (0.06-0.53)
Nonpsychotic mental disorder NOS	0.55 (0.35-0.85)	0.26 (0.14-0.48)	0.42 (0.19-0.94)	0.12 (0.02-0.83)	0.45 (0.11-1.80)
Alcoholism	0.87 (0.71-1.07)	0.64 (0.50-0.81)	0.49 (0.33-0.73)	0.82 (0.53-1.25)	0.88 (0.51-1.49)
SKIN DISEASES					
Non-melanoma skin cancer	1.16 (1.09-1.23)	1.27 (1.19-1.34)	1.26 (1.16-1.38)	1.27 (1.13-1.43)	1.59 (1.35-1.86)
Actinic keratosis	1.11 (1.05-1.18)	1.20 (1.14-1.26)	1.16 (1.07-1.25)	1.26 (1.14-1.40)	1.23 (1.06-1.43)
Atopic dermatitis	0.96 (0.76-1.23)	1.11 (0.90-1.37)	0.98 (0.70-1.38)	1.19 (0.81-1.76)	2.77 (1.89-4.06)
Contact dermatititis and other eczema	0.92 (0.85-0.99)	1.07 (1.00-1.14)	0.99 (0.89-1.10)	1.20 (1.06-1.36)	2.13 (1.83-2.48)
Dermatitis due to substances taken internally	0.99 (0.69-1.43)	1.06 (0.75-1.49)	0.84 (0.47-1.49)	1.24 (0.68-2.27)	4.69 (2.94-7.48)
Bullous skin diseases	0.91 (0.50-1.63)	1.57 (1.01-2.44)	1.18 (0.55-2.54)	0.59 (0.14-2.38)	3.10 (1.27-7.57)
Discoid lupus	0.42 (0.15-1.12)	2.45 (1.61-3.72)	0.76 (0.28-2.07)	1.60 (0.65-3.97)	3.05 (1.13-8.24)
Psoriasis and similar disorders	0.92 (0.77-1.10)	1.31 (1.12-1.52)	1.05 (0.82-1.33)	1.15 (0.85-1.56)	3.55 (2.73-4.60)
Seborrheic keratosis	1.10 (1.02-1.19)	1.08 (1.00-1.16)	1.33 (1.20-1.47)	1.48 (1.30-1.68)	1.09 (0.88-1.36)
Folliculitis and related conditions	0.97 (0.68-1.38)	1.03 (0.73-1.46)	0.35 (0.14-0.84)	1.59 (0.91-2.76)	1.78 (0.88-3.60)
Asteatosis	0.85 (0.66-1.09)	0.99 (0.79-1.24)	1.12 (0.81-1.54)	1.24 (0.85-1.82)	2.24 (1.46-3.43)
Urticaria	0.71 (0.51-0.98)	0.95 (0.73-1.22)	1.26 (0.90-1.78)	1.23 (0.80-1.91)	2.01 (1.22-3.33)
MISCELLANEOUS CONDITIONS					
Benign prostate hyperplasia	1.07 (0.99-1.15)	0.96 (0.89-1.03)	1.08 (0.97-1.20)	1.27 (1.11-1.46)	0.88 (0.72-1.08)
Gastric ulcer	1.00 (0.84-1.19)	0.90 (0.76-1.07)	0.90 (0.70-1.17)	1.53 (1.18-1.99)	0.70 (0.41-1.19)
Decubitus ulcer	0.74 (0.59-0.93)	0.58 (0.45-0.75)	0.62 (0.43-0.91)	0.38 (0.21-0.68)	0.57 (0.28-1.15)
Specified anomalies of spinal cord (myelodysplasia)	1.97 (0.95-4.08)	1.62 (0.76-3.45)	0.53 (0.07-3.88)	2.38 (0.74-7.70)	1.71 (0.24-12.16)
Asphyxia	0.97 (0.78-1.22)	0.88 (0.70-1.11)	0.69 (0.47-1.02)	0.71 (0.44-1.15)	1.41 (0.84-2.36)
Hip fracture	0.88 (0.73-1.06)	0.60 (0.49-0.74)	0.51 (0.36-0.72)	0.75 (0.52-1.09)	0.63 (0.34-1.18)

Supplemental Table 4 notes

Abbreviations: HIV human immunodeficiency virus, HCV hepatitis C virus, NOS not otherwise specified, TIA transient ischemic attack

* Odds ratios are adjusted for sex, age and calendar year of case/control selection, race, and number of provider claims per year. Results are not shown for models that failed to converge. Odds ratios that are shaded were identified as SELECT3 conditions for the indicated NHL subtype, and underlining indicates statistical significance.

Case 3:16-md-02741-VC Document 654-2 Filed 10/28/17 Page 22 of 138

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REVIEW ARTICLE

Evaluation of carcinogenic potential of the herbicide glyphosate, drawing on tumor incidence data from fourteen chronic/carcinogenicity rodent studies

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Abstract

Glyphosate, an herbicidal derivative of the amino acid glycine, was introduced to agriculture in the 1970s. Glyphosate targets and blocks a plant metabolic pathway not found in animals, the shikimate pathway, required for the synthesis of aromatic amino acids in plants. After almost forty years of commercial use, and multiple regulatory approvals including toxicology evaluations, literature reviews, and numerous human health risk assessments, the clear and consistent conclusions are that glyphosate is of low toxicological concern, and no concerns exist with respect to glyphosate use and cancer in humans. This manuscript discusses the basis for these conclusions. Most toxicological studies informing regulatory evaluations are of commercial interest and are proprietary in nature. Given the widespread attention to this molecule, the authors gained access to carcinogenicity data submitted to regulatory agencies and present overviews of each study, followed by a weight of evidence evaluation of tumor incidence data. Fourteen carcinogenicity studies (nine rat and five mouse) are evaluated for their individual reliability, and select neoplasms are identified for further evaluation across the data base. The original tumor incidence data from study reports are presented in the online data supplement. There was no evidence of a carcinogenic effect related to glyphosate treatment. The lack of a plausible mechanism, along with published epidemiology studies, which fail to demonstrate clear, statistically significant, unbiased and non-confounded associations between glyphosate and cancer of any single etiology, and a compelling weight of evidence, support the conclusion that glyphosate does not present concern with respect to carcinogenic potential in humans.

Keywords

amino acid, carcinogenicity, epidemiology, glyphosate, herbicide, mouse, neoplasm, phosphonomethylglycine, Roundup, rat, regulatory, tumor

History

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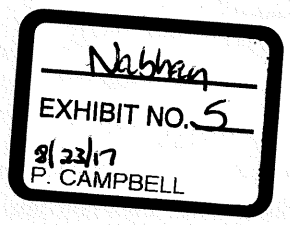

Table of Contents

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Introduction

Glyphosate (Figure 1), an aminophosphonic analog of the natural amino acid glycine, is widely used as an herbicide for the control of annual and perennial grasses and broadleaved weeds. Glyphosate inhibits 5-enolpyruvateshikimate-3-phosphate synthase (EPSPS), an enzyme of the aromatic acid biosynthesis pathway, which is not present in the animal kingdom. Glyphosate-based herbicide formulations (GBFs) were introduced in 1974 and are formulated with

Case 3:16-md-02741-VC Document 654-2 Filed 10/28/17 Page 24 of 138

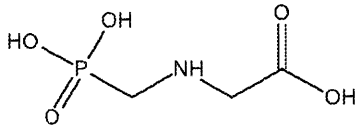

Figure 1. Structure of glyphosate acid.

sodium-, potassium-, ammonium- and isopropyl ammonium-salt forms of the active ingredient. The bulk-manufactured active herbicide glyphosate has the synonyms glyphosate technical acid, technical grade glyphosate and glyphosate acid.

The economic importance of glyphosate for growers is high. It has been estimated that a hypothetical ban of glyphosate would lead to decreases in the production of wheat, fodder, maize and oilseeds, by 4.3–7.1%, with the result of an estimated annual welfare loss of 1.4 billion USD to society in the European Union alone (Schmitz and Harvert 2012). Furthermore, glyphosate plays an important role in integrated pest management strategies, and affords the environmental benefit of substantially reduced soil erosion resulting from of no-till and reduced-till agriculture.

The long-term toxicity and carcinogenicity of glyphosate has been investigated by multiple entities including academia, registrants, and regulatory authorities, and the data generated have been evaluated in support of herbicide regulatory approvals in many world regions including the USA (US EPA 1993) and the European Union (EC 2002), and several scheduled reevaluations are currently ongoing in the USA, Canada, Japan and Europe (Germany Rapporteur Member State 2015a), with imminent conclusions.

Studies of appropriate scientific quality are the basis for regulatory decision making. Mandatory testing guidelines (TGs) exist for toxicological studies submitted for regulatory review of active substances for plant protection in many regions of the world. Such TGs have been released, *inter alia*, by the United States Environmental Protection Agency (US EPA 2012), the European Union (EU 2008), the Japanese Ministry of Agriculture, Forestry and Fisheries (JMAFF 2000), and the Organization of Economic Co-operation and Development (OECD 2012b). These TGs set quality standards for each type of study by giving guidance regarding test species, strains, and number of animals to be used, the choice of dosing, exposure duration, and parameters to be measured and observed, as well as for the reporting of results. Due to the lack of effective legal and regulatory provisions for the sharing of vertebrate study data in the past, and to guarantee the safety of technical glyphosate obtained from different processes of synthesis, several manufacturers of glyphosate had to initiate toxicological testing programs of their own. Occasionally, regulatory studies had to be repeated to reflect major changes in the underlying TG. In the case of glyphosate, this has given rise to a multitude of studies for the same toxicological endpoints, leading to the availability of an extraordinarily robust scientific study database that can be considered unique among pesticides, industrial chemicals, and pharmaceuticals. Such a remarkable volume of studies addressing the same endpoints, conducted over the last 40 years by several independent companies and laboratories while toxicology test guidelines have evolved, warrants investigation for consistency, reliability, and application to their intended purpose: identifying potential human health hazards and setting appropriate endpoints for human health risk assessment. Studies conducted with equivalent test substances using the same TG are readily comparable and can be evaluated by regulators following standardized schemes. Minor differences in the findings reported by such repetitive studies are attributable to statistical chance, natural biological variability, type of basal diet, rate of feed consumption, animal strain differences, choice of dose levels, inter-strain genetic drift over time due to varying vendor breeding practices, changes in animal care and husbandry practices across laboratories over the years, inter-laboratory variations in clinical measurements, and differences between individual pathologist evaluation and interpretation of tissue specimens.

Glyphosate is under significant political pressure due to its widespread use, particularly in association with use on genetically modified crops. One focus area of contention has been the human safety of glyphosate, which has been repeatedly challenged by interest groups via the media, as well as select research publications in the scientific literature (Antoniou et al. 2012, Aris and Leblanc 2011, Aris and Paris 2010, Benachour and Seralini 2009, Gasnier et al. 2010, Paganelli et al. 2010, Romano et al. 2012, Romano et al. 2010). To that end, one specific publication by Seralini et al. (2012, retracted) drew significant criticism from both the toxicology and broader scientific communities (Barale-Thomas 2013, Berry 2013, de Souza and Oda 2013, Grunewald and Bury 2013, Hammond et al. 2013, Langridge 2013, Le Tien and Le Huy 2013, Ollivier 2013, Panchin 2013, Sanders et al. 2013, Schorsch 2013, Tester 2013, Trewavas 2013, Tribe 2013). After a special review of the investigators' raw data by a mutually agreed-upon expert panel, the manuscript was retracted by *Food and Chemical Toxicology* (FCT), for reasons of inconclusive data and unreliable conclusions (Hayes 2014). The Editor of the *International Journal of Toxicology* highlighted this manuscript as an example of possible failure of the peer review process in a well-respected toxicology journal with an editorial board of well-known and respected toxicologists (Brock 2014). The manuscript was later republished without peer-review in an open access journal (Seralini et al. 2014), but will not be addressed in this data evaluation due to the inappropriate study design, insufficient reporting of tumor incidence data, and the lack of a data supplementary to the manuscript.

The chronic/carcinogenicity studies discussed in this paper have been submitted to and evaluated by a variety of agencies over time, including the World Health Organization (WHO/FAO 2004b, WHO/FAO 2004a), the United States Environmental Protection Agency (US EPA 1993), the European Rapporteur Member State Germany for the initial glyphosate Annex I listing (EC 2002) and the recent European re-evaluation (Germany Rapporteur Member State 2015a), as well as the ongoing reevaluations in the USA, Canada and Japan. These regulatory bodies, drawing upon internal and/or external expertise, have consistently concluded that glyphosate is devoid of carcinogenic risk to humans.

The purpose of this article is to provide the broader scientific community with insight into this large body of carcinogenicity data on glyphosate, originally generated for

Case 3:16-md-02741-VC Document 654-2 Filed 10/28/17 Page 25 of 138

regulatory purposes. Each study discussed in this review has been assigned a reliability score in Tables 3–19, following the Klimisch scoring system (Klimisch et al. 1997). In this system, a score of 1 is assigned to studies that are fully reliable based on compliance with Good Laboratory Practice (GLP) and adherence to appropriate study guidelines. A score of 2 is appropriate if some guideline requirements are not met, but if these deficiencies do not negatively affect the validity of the study for its regulatory purpose. Studies with a reliability of 3 employ a test design that is not fit for the scientific purpose of the study, due to significant scientific flaws, or the objective of the study not covering the regulatory endpoints, or both. Such studies can provide supplemental information but do not allow a stand-alone appraisal of a regulatory endpoint. No studies were assigned a reliability of 4, since each report contained sufficient information to judge the validity of the study.

This manuscript presents the robust glyphosate carcinogenicity data generated by industry. Study summaries will focus on carcinogenicity evaluation, to allow third parties the opportunity to independently evaluate the carcinogenicity data presented alongside other relevant data on carcinogenicity, i.e. genotoxicity testing and epidemiology, and facilitate a multidisciplinary carcinogenicity assessment as proposed in the literature, by recognized experts in the fields of toxicology and human health risk assessment (Adami et al. 2011).

Absorption, distribution, metabolism and excretion of glyphosate

A number of absorption, distribution, metabolism, and excretion studies (ADME) have been conducted on glyphosate for evaluation in regulatory submissions (EC 2002, US EPA 1993, WHO/FAO 2004a) and also by academic institutions (Anadon et al. 2009). Glyphosate consistently demonstrates low gastrointestinal absorption (20–40%). Its metabolism is very limited, whereby only small quantities of a single metabolite, aminomethylphosphonic acid (AMPA), are eliminated in feces. AMPA is likely produced by the limited metabolism of glyphosate by the gastrointestinal microflora, rather than via mammalian metabolism. Glyphosate is structurally akin to a phase II metabolite, a glycine-conjugate of methyl phosphonate, and thus avails itself to rapid urinary excretion. Systemic elimination is biphasic, with alpha-phase half-lives in the range of 6–14 h (Anadon et al. 2009, WHO/FAO 2004a).

Toxicological properties of glyphosate

Table 1 contains a short overview of toxicological endpoints of glyphosate that have been published in the List of Endpoints identified for glyphosate by the Rapporteur in the European Union under Regulation 1107/2009 (Germany Rapporteur Member State 2015c). Glyphosate is of low acute toxicity via all routes of exposure. Glyphosate's active ingredient, an organic acid, has an irritating effect on mucosa which is evidenced by eye irritation and effects on oral and gastrointestinal mucosa; final formulated products contain more neutral pH salt forms, as reflected in the tabulated eye irritation data reported in Table 11, on page 109 of the 2004 JMPR Toxicological Evaluation (WHO/FAO 2004a). Glyphosate is not mutagenic, not neurotoxic, and has no effect on pre-natal development and fertility at doses not exceeding the maximum tolerated dose (MTD).

Genotoxicity

Very recently, a review of the vast body of genotoxicity studies on glyphosate and GBFs has been published (Kier and Kirkland 2013), including an online data supplement presenting detailed data from 66 separate *in vitro* and *in vivo* genotoxicity assays. The authors incorporated these studies and published genotoxicity data into a weight-of-evidence analysis. The vast majority (over 98%) of the available bacterial reversion and *in vivo* mammalian micronucleus and chromosomal aberration assays were negative. Negative results for *in vitro* gene mutation and a large majority of negative results for clastogenic effect assays in mammalian cells support the conclusion that glyphosate is not genotoxic for these endpoints in mammalian test systems. DNA damage effects are reported in some instances for glyphosate at high or toxic dose levels. The compelling weight of evidence is that glyphosate and typical GBFs are negative in core assays, indicating that the reported high-dose effects are secondary to toxicity and are not due to DNA-reactive mechanisms. Mixed results were observed for micronucleus assays in non-mammalian systems and DNA damage assays of GBFs. These effects of GBFs may also be associated with surfactants present in the formulated products. Kier and Kirkland conclude that glyphosate and its typical formulations do not present significant genotoxic risk under normal conditions of human or environmental exposures.

Epidemiology

Available epidemiological studies of glyphosate and cancer endpoints were recently reviewed (Mink et al. 2012). Seven cohort studies and fourteen case-control studies examining a potential association between glyphosate and one or more cancer outcomes were subjected to a qualitative analysis. The review found no consistent pattern of positive associations between total cancer (in adults or children) or any site-specific cancer, and exposure to glyphosate. A recent review article (Alavanja et al. 2013) cites one epidemiology study associating glyphosate use with non-Hodgkin's lymphoma (NHL), and accepts the study findings *prima facie*. However, Alavanja et al. (2013) did not highlight six other published epidemiology studies which evaluated glyphosate use and NHL, noting that any association between NHL and glyphosate use was null or not statistically significant. All seven studies were scrutinized by Mink et al. (2012). NHL is not a specific disease, as mentioned in both the epidemiology review publications above, but is rather multiple presentations of lymphoma which are simplistically classified as not being Hodgkin's lymphoma (HL). This dichotomous classification of HL/NHL was rejected by the World Health Organization in 2001, whereby 43 different lymphomas of various etiologies were precisely characterized (Berry 2010). The Bradford Hill criteria are often applied in efforts to determine whether an association between a health effect and human exposure may be deemed causal. However, an important premise often overlooked from Sir Austin Bradford Hill's famous speech of 1965, is that before applying these criteria, the observations should "reveal an association between two variables, perfectly clear-cut and beyond what we care to attribute to the play of chance" (Bradford Hill 1965). This predicate of the association being "perfectly clear-cut"

Case 3:16-md-02741-VC Document 654-2 Filed 10/28/17 Page 26 of 138

Table 1. Summary of toxicological endpoints for glyphosate (Germany Rapporteur Member State 2015c).

Endpoint	Value	Remark
Oral absorption	*ca* 20%	Rat, *in vivo*
Dermal absorption	< 1%	Human, *in vitro*, 0.015 g glyphosate/L
Rat LD50 oral	> 2000 mg/kg bw	
Rat LD50 dermal	> 2000 mg/kg bw	
Rat LC50 inhalation	> 5 mg/L	4-h exposure
Skin irritation	Not irritating	
Eye irritation	Acid: moderately to severely irritating Salts: slight or non-irritating	
Skin sensitization	Not sensitizing (LLNA, Magnusson-Kligmant, and Buehler test)	
Genotoxicity	Not genotoxic (*in vitro* and *in vivo*)	
Chronic toxicity	BW gain, liver (organ weight ↑, clinical chemistry, histology); salivary glands (organ weight ↑, histology); stomach mucosa and bladder epithelium(histology); eye (cataracts), caecum (distention, organ weight ↑) NOAEL = 100 mg/kg bw/day (2-yr rat)	Critical study used for ADI setting
Reproductive toxicity	Reduced pup weight at parentally toxic doses. NOAEL = 300 mg/kg bw/day	
Developmental toxicity	Post-implantation loss, fetal BW & ossification ↓: effects confined to maternally toxic doses Rat NOAEL: 300 mg/kg bw/day Rabbit NOAEL: 50 mg/kg bw/day	
Delayed neurotoxicity	No relevant effects, NOAEL: 2000 mg/kg bw/day	
Acceptable Daily Intake (ADI)	0.5 mg/kg bw/day Based on developmental toxicity in rabbits	Safety factor 100
Acceptable Operator Exposure Level (AOEL)	0.1 mg/kg bw/day Based on maternal toxicity in rabbit teratogenicity study	Safety factor 100 Corrected for oral absorption of 20%

was recently highlighted as requiring statistical significance, wherein the confidence interval of a relative risk ratio is bracketed above 1.0, as well as concluding that the association may not be attributable to bias, confounding or sampling error (Woodside and Davis 2013). According to Bradford Hill, should an epidemiology study be considered to demonstrate a "perfectly clear-cut" association between glyphosate exposure and a human health outcome, only then should the Bradford Hill criteria be investigated to determine whether there is causality. To date, no such "perfectly clear-cut" association between glyphosate exposure and any cancer exists. However, investigative toxicology is an important discipline to evaluate chemicals before any human exposure occurs, and these data may inform subsequent considerations of whether associations are attributable to causality. One Bradford Hill criterion in establishing disease causality is plausibility, based on known disease etiologies. In the case of lymphoma, there are numerous etiologies for the numerous and different lymphoma diseases, and as such, each lymphoma type should be investigated for a plausible mechanism to determine whether causality may be attributed an appropriately qualified association. Another Bradford Hill criterion is identification of a biological gradient, or dose-response, which is a key consideration in the following data evaluation.

Chronic toxicity studies

Several one-year chronic studies have been undertaken in dogs and one in rats, in addition to the many chronic/carcinogenicity studies with one-year interim sacrifice groups. Current Test Guidelines (OECD, EPA, EU and JMAFF) for long-term studies clearly state that the highest dose tested should either be at the maximum tolerated dose (MTD), conventionally interpreted as a dose causing non-lethal toxicity, often noted as reduced body weight gain of 10% or more (IUPAC 1997). For test substances with low toxicity, a top dose not exceeding 1000 mg/kg bw/day may apply, except when human exposure indicates the need for a higher dose level to be used (OECD 2012a). All human exposure estimates are well below 1 mg/kg bw/day (see Discussion section), so that 1000 mg/kg bw/day is a practical limit dose for glyphosate in carcinogenicity studies. In the original pre-guideline chronic/carcinogenicity study, rats were dosed well below the MTD (Monsanto 1981), but in many subsequent studies, they were dosed well in excess of today's standard practice of not exceeding the dose limit.

Dog chronic studies

Five one-year oral toxicity studies have been conducted in Beagle dogs (Table 2). Studies in dogs are not designed to detect neoplastic effects; these studies are therefore not discussed in detail. Nonetheless, the histopathological investigations that are part of one-year dog studies according to OECD TG 452 did not identify (pre) neoplastic lesions related to the administration of glyphosate.

Treatment-related effects in dog studies with glyphosate were restricted to non-specific findings like small retardations in body weight gain and soft stools, which are common findings in this test species. The lowest relevant NOAEL (i.e. highest NOAEL below the lowest LOAEL) in dogs on a daily treatment regimen for one year was 500 mg/kg bw/day. These studies demonstrate that glyphosate is of very low toxicity following repeat exposures in dogs.

Rat chronic studies

The chronic toxicity potential of glyphosate acid was assessed in a 12-month feeding study (conducted in 1995 and 1996) in

Table 2. Summary of one-year toxicity studies with glyphosate.

Authors:	Monsanto (1985)
Reliability/Justification	2 Study performed according to GLP and OECD guideline requirements, with the following deviation: MTD not reached by highest dose
Substance:	Glyphosate (96.1% pure)
Species/Strain:	Dog/Beagle, groups of 6 ♂ and 6 ♀
Administration route:	Oral, capsule
Doses:	0, 20, 100, 500 mg/kg bw/day
Duration:	1 year
Findings:	≥ 500 mg/kg bw/day: NOAEL (♂ + ♀) no treatment-related effects
Authors:	**Cheminova (1990)**
Reliability/Justification	1 Study performed according to GLP and OECD guideline requirements, with no deviations.
Substance:	Glyphosate (98.6–99.5% pure)
Species/Strain	Dog/Beagle, groups of 4 ♂ and 4 ♀
Administration route:	Oral, capsule
Doses:	0, 30, 300, 1000 mg/kg bw/day
Duration:	1 year
Findings:	300 mg/kg bw/day: NOAEL (♂ + ♀) 1000 mg/kg bw/day: soft, liquid stools (attributable to capsule administration): equivocal impact on body weight gain
Authors:	**Nufarm (2007)**
Reliability/Justification	2 Study performed according to GLP and OECD guideline requirements, with the following deviation: MTD not reached by highest dose
Substance:	Glyphosate (95.7% pure)
Species/Strain	Dog/Beagle, groups of 4 ♂ and 4 ♀
Administration route:	Oral, capsule
Doses:	0, 30, 125, 500 mg/kg bw/day
Duration:	1 year
Findings:	≥ 500 mg/kg bw/day: NOAEL (♂ + ♀) No treatment-related effects
Authors:	**Arysta Life Sciences (1997c)**
Reliability/Justification	2 Study performed according to GLP and OECD guideline requirements, with the following deviation: MTD not reached by highest dose
Substance:	Glyphosate (94.6% pure)
Species/Strain	Dog/Beagle, groups of 4 ♂ and 4 ♀
Administration route:	Oral, diet
Concentration:	0, 1600, 8000, 50 000 ppm diet (♂ about 34.1, 182, 1203 mg/kg bw/day; ♀ about 37.1, 184, 1259 mg/kg bw/day)
Duration:	1 year
Findings:	182/184 mg/kg bw/day: NOAEL (♂/♀) At high dose: loose stool, non-statistically significant retarded body weight gain, decreased urinary pH, slight and non-statistically significant focal pneumonia (♀), minor clinical chemistry changes of Cl ↑, albumin ↓, P ↓ (♀)
Authors:	**Syngenta (1996a)**
Reliability/Justification	1 Study performed according to GLP and OECD guideline requirements, with no deviations.
Substance:	Glyphosate (95.6% pure)
Species/Strain	Dog/Beagle, groups of 4 ♂ and 4 ♀
Administration route:	Oral, diet
Concentration:	0, 3000, 15 000, 30 000 ppm diet (♂ about 90.9, 440, 907 mg/kg bw/day; ♀ about 92.1, 448, 926 mg/kg bw/day)
Duration:	1 year
Findings:	15 000 ppm diet: NOAEL (♀) ≥ 30 000 ppm diet: NOAEL (♂): No treatment-related effects 30 000 ppm diet: slight body weight reduction (♀)
Authors:	**Syngenta (1996b)**
Reliability/Justification	1 Study performed according to GLP and OECD guideline requirements, with no deviations.
Substance:	Glyphosate (95.6% pure)
Species/Strain	Rat/Wistar Alpk: AP$_f$SD, groups of 24 ♂ and 24 ♀
Administration route:	Oral, diet
Concentration:	0, 2000, 8000, 20 000 ppm diet (♂ about 141, 560, 1409 mg/kg bw/day; ♀ about 167, 671, 1664 mg/kg bw/day)
Duration:	1 year
Findings:	8000 ppm diet: NOAEL (♂+♀) 20 000 ppm diet: parotid salivary glands (focal basophilia of the acinar cells considered non-adverse adaptive response, ♂: 13/24, ♀: 15/24), body weight reduction

24 male and female Wistar rats per group, dosed at 0, 2000, 8000 and 20 000 ppm (Syngenta 1996). The mean achieved dose levels were 0, 141, 560 and 1409 mg/kg bw/day for males, and 0, 167, 671 and 1664 mg/kg bw/day for females. Spastically significant reductions in bodyweight were evident in animals receiving 20 000 ppm glyphosate acid, together with a marginal reduction in bodyweight in rats receiving 8000 ppm, but food consumption relative to controls was lower for these dose groups, suggesting reduced palatability of the diets containing these doses of glyphosate. There were no toxicologically significant or treatment-related effects on hematology, blood and urine clinical chemistry, or organ weights (Table 2).

The treatment-related pathological finding, that is increased incidence of mild focal basophilia, and a hypertrophy of the acinar cells of the parotid salivary gland in both sexes which had received 20 000 ppm glyphosate acid, is considered an adaptive response due to oral irritation from the ingestion of glyphosate, an organic acid, in the diet. This was verified by

Case 3:16-md-02741-VC Document 654-2 Filed 10/28/17 Page 28 of 138

mode of action investigations and studies with dietary administration of citric acid, a non-toxic organic acid with irritation properties and pH dilution curve similar to those of glyphosate (Saltmiras et al. 2011), which elicited the same response in the acinar cells of the parotid salivary glands.

In conclusion, the 12-month NOAEL in rats for glyphosate acid, as determined from this study, is 8000 ppm (corresponding to 560 mg/kg bw/day in males and 671 mg/kg bw/day in females). This study does not cover neoplastic endpoints. These were addressed in a subsequent study by the same sponsor (Syngenta 2001). Consistent with the findings observed in dogs, this study demonstrates that glyphosate is of very low toxicological concern following long-term daily exposures.

Similarly, most of the following 2-year rat carcinogenicity studies included additional groups for 1-year interim sacrifice to evaluate chronic toxicity. These studies did not elucidate significant toxicological concerns for chronic dietary exposures to glyphosate in rats in multiple expert reviews by governmental agencies and several technical branches of the World Health Organization including the Joint Meeting on Pesticide Residues Toxicological Evaluations (WHO/FAO 2004a).

Carcinogenicity studies

Chronic/carcinogenicity tests are designed to simulate lifetime exposures to an individual chemical and represent the most robust *in vivo* assay to evaluate the effects of chronic exposure including carcinogenicity. These models are biological systems with natural background variability due to tumor formation as a natural consequence of aging. Glyphosate was found to have no carcinogenic potential, which is reflected in the data showing only background noise of spontaneous tumors across the wide range of doses. Normal biological variability should display various tumor types across all dose groups without an apparent dose-response. The study summaries discuss "select neoplasms", identified by the authors as having an elevated incidence above concurrent controls across one or more dose groups, most of which lacked statistical significance and/or dose-response within an individual study. These tumors are then evaluated in the context of the whole data set, to provide a robust weight of evidence overview for the doses spanning several orders of magnitude. While not all studies have select neoplasms identified in the individual study summary tables, select neoplasms for all studies are reported in Tables 20–23. Summary tables of the select neoplasms footnote the strain tested for each dose, to allow consideration of strain differences in spontaneous tumor susceptibility (Tables 20–23). In addition, complete tumor incidence summary tables have been extracted from the original eight rat (the published rat study, Study 9, is not included) and five mouse study reports or study files, and posted in their original format, as a comprehensive online data supplement to this manuscript.

Rat carcinogenicity

A total of nine chronic/carcinogenicity studies in the rat, including one peer-reviewed published study, were available for review. This duplication of large-scale studies in the same animal model using the same test substance is not consistent with today's broader appreciation for animal welfare and the reduction of unnecessary animal testing. However, these studies offer the opportunity for a critical discussion of findings in individual studies in the context of the larger body of data. Wistar and Sprague Dawley were the strains used for the bioassays in rats. Seven studies were conducted under conditions of GLP, and two studies were not under GLP (Study 1, conducted before the introduction of GLP; Study 9, non-GLP). Most studies in rats were designed as combined chronic toxicity/carcinogenicity studies, with interim sacrifices after 12 months of treatment for the assessment of non-neoplastic chronic toxicity. Statistical methods are noted in the manuscript tables where statistical significance was attained. Statistical differences in neoplasm incidence summary tables are reported in the online data supplements. Chronic endpoints and NOAEL values are captured in each study summary table; however, the following study reviews focus on carcinogenicity.

Study 1 (Monsanto 1981)

An early study into the long-term effects of orally administered glyphosate in the rat was conducted between 1978 and 1980 (Monsanto 1981), prior to the adoption of international test guidelines and GLP standards (Tables 3–6). Nonetheless, the test protocol was broadly compliant with OECD TG 453 (1981). However, an MTD was not reached and the high dose was well below an acceptable dose limit of 1000 mg/kg bw/day. Therefore, this study is rated Klimisch 3 for reliability, and is considered inadequate for carcinogenicity evaluation from a regulatory perspective.

Groups of 50 male and 50 female Sprague Dawley rats were administered glyphosate acid in the diet, at concentrations of 0, 30, 100 and 300 ppm, for up to least 26 months. The mean doses achieved were 0 (control), 3, 10, and 31 mg/kg bw/day for the males, and 0 (control), 3, 11, and 34 mg/kg bw/day for the females. Study results are summarized in Table 3.

In general, the incidences of all neoplasms observed in the treated and control animals were similar, or occurred at low incidence, such that a treatment-related association could not be made. The most common tumors found were common spontaneous neoplasms, as reported in the literature relating to rat (Johnson and Gad 2008), in the pituitary glands of both control and treated animals (Table 4). In the females, mammary gland tumors were the next most common neoplasm across control and dose groups (see data Supplementary Study 1 to be found online at http://informahealthcare.com/doi/abs/10.3109/10408444.2014.1003423).

Table 3. Study 1–26-month feeding study of glyphosate in rats (Monsanto 1981).

Study owner:	Monsanto (1981)
Reliability/Justification:	3 Study not performed under GLP. High-dose well below MTD. Does not conform to modern testing standards.
Substance:	Glyphosate (98.7% pure)
Species/Strain:	Rat/Sprague-Dawley, groups of 50 ♂ and 50 ♀
Administration route:	Diet
Concentration:	0, 30, 100, 300 ppm diet (♂ about 0, 3, 10, 31 mg/kg bw/day: ♀ about 0, 3, 11, 34 mg/kg bw/day)
Duration:	26 months
Findings:	≥ 300 ppm diet: NOAEL (♂ + ♀) No treatment-related effects
Select neoplasms:	Pituitary adenoma, Testes interstitial cell

Table 4. Study 1 – Pituitary tumor findings.

	Dose group (mg/kg bw/day)							
	Males				Females			
Tumors	0	3.05	10.3	31.49	0	3.37	11.22	34.02
Pituitary tumors	Number of animals/total number examined (% per group)							
Adenomas - B	16/48 (33)	19/49 (39)	20/48 (42)	18/47 (38)	34/48 (70)	29/48 (60)	31/50 (62)	26/49 (53)
Carcinomas - M	3/48 (6)	2/49 (4)	3/48 (6)	1/47 (2)	8/48 (17)	7/48 (14)	5/48 (19)	12/49 (24)
Combined	19/48 (40)	21/49 (43)	23/48 (48)	19/47 (40)	42/48 (88)	36/48 (75)	36/50 (72)	38/49 (78)

B benign, *M* malignant

The incidence of interstitial cell tumors of the testes in male rats in both the scheduled terminal sacrifice animals, as well as for all animals, suggested a possible treatment-related finding, and was presented along with contemporary historical control data for comparison (Tables 5 and 6). It was noted that at 12 months, the incidence of interstitial tumors was near zero; however, in animals aged 24–29 months at necropsy, the incidence increased to approximately 10%. The historical control data for chronic toxicity and carcinogenicity from 5 studies terminated at 24–29 months showed background levels of interstitial cell tumors comparable to those found at the highest dose in the study. Furthermore, the reported incidences in all dose groups reflect the normal range of interstitial cell tumors in rat testes, reported in the Registry of Industrial Toxicology Animal Data (Nolte et al. 2011). The incidence of interstitial cell hyperplasia did not provide evidence of a pre-neoplastic lesion. The investigators noted that at terminal sacrifice, the incidence of interstitial cell tumor was 15.4% (4/26), while the range in control animals from 5 contemporary studies (historical controls) was 6.2% (4/65) to 27.3% (3/11), with an overall mean value of 9.6% (16/166). When all animals on test are included, the incidence for the high-dose males was 12% (6/50), compared to a contemporary historical control range of 3.4% (4/116) to 6.7% (5/75), with a mean of 4.5% (24/535). The concurrent control incidence of interstitial cell tumors (0%) was not representative of the normal background incidence noted in contemporary historical control data. Therefore, the data suggest that the incidence in treated rats is within the normal biological variation observed for interstitial cell tumors at this site in this strain of rat. When evaluated in the context of the full data set for male rats (Table 20), a dose-response is clearly absent for the 25 doses evaluated in rats, ranging from 3 to 1290 mg/kg bw/day, which demonstrates that this tumor is clearly not a consequence of glyphosate exposure.

In conclusion, glyphosate was not considered carcinogenic in Sprague Dawley rats following continuous dietary exposure of upto 300 ppm, corresponding to 31 and 34 mg/kg bw/day in males and females, respectively, which is consistent with evaluations by the US EPA (US EPA 1993), the original Annex I listing in Europe (EC 2002), and WHO/FAO (WHO/FAO 2004a).

Based on the low doses tested in Study 1, Monsanto was obliged to conduct a second chronic/carcinogenicity study in rats (Study 2, discussed below) in accordance with OECD TG 453 (1981), which had been developed and instituted after this initial study was conducted.

Study 2 (Monsanto 1990)

In response to evolving regulatory requirements, this study was conducted in accordance with the contemporary version of OECD TG 453 (Monsanto 1990). The chronic toxicity and carcinogenic potential of glyphosate were assessed in a 24-month feeding study in 50 male and 50 female Sprague Dawley rats, dosed with 0, 2000, 8000 and 20 000 ppm (equivalent to mean achieved dose levels of 0, 89, 362 and 940 mg/kg bw/day for males and 0, 113, 457 and 1183 mg/kg bw/day for females (Table 7). In addition, 10 rats per sex per dose were included for interim sacrifice after 12 months. Observations covered clinical signs, ophthalmic examinations, body weight, food consumption, hematology, clinical chemistry and urinalysis, as well as organ weights, necropsy, and histopathological examination. This study was rated Klimisch 1 for reliability.

Treatment-related findings in this study were significantly reduced body weight in high-dose females, as well as increased liver weight in high-dose males and females, and a slight increase in incidence of cataract lens changes in high-dose males, which was not statistically significant for eye lesions confirmed by histopathology (Table 7). The body weight changes confirm that the MTD was achieved in the highest dose group. Benign thyroid C-cell adenomas were statistically higher than controls in the mid-dose terminally sacrificed males, but when pooled with unscheduled deaths, no statistically significant increase was noted. Benign pancreas islet cell adenomas were not statistically higher for the unscheduled or scheduled deaths, but when combined, were statistically higher than controls in the low and high dose males. In both cases, the benign tumors did not exhibit a dose-response, and did not progress to carcinomas, and thus the US EPA concluded that these tumors were not related to the administration

Table 5. Study 1 - Interstitial cell tumor findings in the testes.

	Dose (mg/kg bw/day)			
Tumors	0	3.05	10.3	31.49
Interstitial cell tumor – B	Number of animals/total number examined (% per group)			
Terminal sacrifice	0/15 (0)	2/26 (7.7)	1/16 (6.3)	4/26 (15.4)
All Animals	0/50 (0)	3/50 (6)	1/50 (2)	6/50 (12)
Interstitial cell hyperplasia	Number of animals (% per group)			
Terminal sacrifice	1/15 (6.7)	1/26 (3.8)	0/16 (0)	0/26 (0)
All Animals	1/50 (2)	1/50 (2)	1/50 (2)	0/50 (0)

B benign, *M* malignant

Table 6. Study 1 – Summary of the contemporary historical control data for interstitial cell tumors in the testes of rats in chronic toxicity studies.

	Study 1	Study 2	Study 3	Study 4	Study 5	
	Number of control animals/total number examined (% per study)					Range
Terminal sacrifice	4/65 (6.2)	3/11 (27.3)	3/26 (11.5)	3/24 (12.5)	3/40 (7.5)	6.2–27.3%
All animals	4/116 (3.4)	5/75 (6.7)	4/113 (3.5)	6/113 (5.3)	5/118 (4.2)	3.4–6.7%

of glyphosate (US EPA 1993). These neoplasms, in addition to skin keratoacanthoma in males, a common rat tumor, were selected for further weight of evidence evaluation (Tables 20 and 21). No evidence of a glyphosate-induced carcinogenic effect was noted in either sex (see data Supplementary Study 2 to be found online at http://informahealthcare.com/doi/abs/10.3109/10408444.2014.1003423).

In conclusion, glyphosate was not carcinogenic in Sprague Dawley rats following continuous dietary exposure of up to 20 000 ppm for 24 months, corresponding to 940 and 1183 mg/kg bw/day in males and females, respectively, which is consistent with evaluations by the US EPA (US EPA 1993), European Authorities (EC 2002), and WHO/FAO (WHO/FAO 2004a).

Study 3 (Cheminova 1993a)

The chronic toxicity and carcinogenic potential of glyphosate technical acid were assessed in a 104-week feeding study in male and female Sprague Dawley rats (Cheminova 1993a). The study was conducted between 1990 and 1992. Groups of 50 rats per sex received daily dietary doses of 0, 10, 100, 300, or 1000 mg/kg bw/day of glyphosate technical acid for 24 months (Table 8). Five additional groups of 35 rats per sex, receiving daily dietary doses of, 0, 10, 100, 300 or 1000 mg/kg bw/day, were included for interim sacrifice at the 12th month for evaluation of chronic toxicity. The dietary glyphosate levels were adjusted weekly to ensure that animals were receiving the intended dose levels at all times. This study was rated Klimisch 1 for reliability.

At 1000 mg/kg bw/day, female mean liver weights were decreased, while males and females had statistically significant reductions in body weight throughout the study, confirming that the MTD was achieved (Table 8). Neoplasms were noted in control and treated groups, but dose-responses were not evident, and no statistically significant increases versus controls were noted for any tumor type ($p < 0.05$). No treatment-related neoplastic lesions were observed at termination,

Table 7. Study 2 – Two-year feeding study of glyphosate in rats (Monsanto 1990).

Study owner:	Monsanto (1990)			
Reliability/Justification:	1 Study performed according to GLP and OECD guideline requirements, with no deviations.			
Substance:	Glyphosate (96.5% pure)			
Species/Strain:	Rat/Sprague-Dawley, groups of 50 ♂ and 50 ♀ (10 rats per sex per dose were included for interim sacrifice after 12 months).			
Administration route:	Diet			
Concentration:	0, 2000, 8000, 20 000 ppm diet (♂ about 0, 89, 362, 940 mg/kg bw/day; ♀ about 0, 113, 457, 1183 mg/kg bw/day)			
Duration:	2 years			
Findings:	8000 ppm diet: NOAEL (♂+♀) 20 000 ppm diet: cataracts (♂), >20% reduced cumulative body weight gain through months 18–20 (♀), 13% increased liver weight (♂). Local effects: inflammation of gastric mucosa			
Select neoplasms:	Pancreatic islet cell adenoma, skin keratoacanthoma (males), thyroid C cell adenoma			
Tumor		Dose (mg/kg bw/day)		
Males	0	89	362	940
Findings for dead and moribund sacrificed animals				
Pancreas: Islet call adenoma – B	1/34 (3%)	4/28 (14%)	2/33 (6%)	4/32 (13%)
Skin: Keratoacanthoma – B	0/36	1/31 (3%)	2/33 (6%)	1/32 (3%)
Thyroid: C cell adenoma – B	0/36	2/29 (7%)	1/31 (3%)	1/33 (3%)
Thyroid: C cell carcinoma – M	0/36	1/29 (3%)	2/31 (6%)	1/33 (3%)
Findings for animals sacrificed at termination				
Pancreas: Islet call adenoma – B	0/14	4/19 (21%)	3/17 (6%)	3/17 (6%)
Skin: Keratoacanthoma – B	0/13	2/19 (11%)	2/17 (12%)	2/17 (12%)
Thyroid: C cell adenoma – B	0/14	2/19 (11%)	*7/17 (41%)	4/17 (24%)
Thyroid: C cell carcinoma – M	0/14	0/19	0/17	0/17
Females	0	113	457	1183
Findings for dead and moribund sacrificed animals				
Pancreas: Islet call adenoma – B	3/28 (11%)	0/28	3/33 (9%)	0/31
Thyroid: C cell adenoma – B	0/28	0/28	1/33 (3%)	2/32 (6%)
Thyroid: C cell carcinoma – M	0/28	0/28	1/33 (3%)	0/32
Findings for animals sacrificed at termination				
Pancreas: Islet call adenoma – B	2/22 (9%)	1/22 (5%)	1/17 (6%)	0/18
Thyroid: C cell adenoma – B	2/22 (9%)	2/22 (9%)	5/17 (29%)	4/18 (22%)
Thyroid: C cell carcinoma – M	0/22	0/22	0/17	0/18

B benign. *M* malignant

*Statistically higher than controls ($p < 0.05$, Fisher's Exact Test with the Bonferroni Inequality).

Case 3:16-md-02741-VC Document 654-2 Filed 10/28/17 Page 31 of 138

Table 8. Study 3 – Two-year feeding study of glyphosate in rats (Cheminova 1993a).

Study owner:	Cheminova (1993a)
Reliability/ Justification:	1 Study performed according to GLP and OECD guideline requirements, with no deviations.
Substance:	Glyphosate (98.7–98.9% pure)
Species/Strain:	Rat/Sprague-Dawley, groups of 50 ♂ and 50 ♀ (additional groups of 35 ♂ and 35 ♀per dose were included for 1-year interim sacrifice)
Administration route:	Diet
Achieved dose:	♂+♀: 0, 10, 100, 300, 1000 mg/kg bw/day (weekly adjustment of dietary concentration for the first 13 weeks and 4-weekly thereafter)
Duration:	2 years
Findings:	300 mg/kg bw/day: NOAEL (♂+♀) 1000 mg/kg bw/day: body weights ↓, urinary pH ↓, salivary glands (histopathology, organ weight ↑); evidence of weak liver toxicity (alkaline phosphatase ↑, ♀: organ weight ↓)
Select neoplasms:	No neoplasms from this study were identified for further consideration.

and no select neoplasms were identified in this study for further consideration (see data Supplementary Study 3 to be found online at http://informahealthcare.com/doi/abs/10.3109/10408444.2014.1003423). Glyphosate was not considered carcinogenic in male and female Sprague Dawley rats following 104 weeks of continuous dietary exposure of up to 1000 mg/kg bw/day, the limit dose, which is consistent with evaluations by the European Authorities (EC 2002, Germany Rapporteur Member State 2015b) and WHO/FAO (WHO/FAO 2004a).

Study 4 (Feinchemie Schwebda 1996)

A 2-year bioassay in the Wistar rat used dietary glyphosate levels of 0, 100, 1000, and 10 000 ppm (Feinchemie Schwebda 1996). Groups of 50 rats per sex were fed for 24 months. The mean achieved dose levels were 0, 7.4, 73.9, and 740.6 mg/kg bw/day (Table 9). This study was rated Klimisch 1 for reliability.

In addition, one vehicle control with ten rats per sex and one high dose (10 000 ppm) group with 20 rats per sex were included for interim sacrifice after one year of treatment, to study non-neoplastic histopathological changes. The mean achieved dose level in the treated group was 764.8 mg/kg bw/day. Observations covered clinical signs, body weight, food consumption, hematology, clinical chemistry, and urinalysis, as well as organ weights, necropsy, and histopathological examination.

There were no treatment-related deaths or clinical signs in any of the dose-groups. Moreover, there were no treatment-related effects on body weight gain or food consumption noted. This suggests that the MTD may not have been reached by the applied dosing regimen.

There was some background variation in the incidences of benign tumors (e.g. reduced tumor incidence in low and mid-dose males, increased tumor incidence in middose females), which was considered incidental in absence of a dose-response relationship (see data Supplementary Study 4 to be found online at http://informahealthcare.com/doi/abs/10.3109/10408444.2014.1003423).

The different liver tumors observed in the dead and moribund sacrificed and terminally sacrificed rats included hepatocellular adenoma, intrahepatic bile duct adenomas, cholangiocarcinoma, hepatocellular carcinoma, histiocytic sarcoma, fibrosarcoma, and lymphosarcoma. Among these, hepatocellular adenomas and carcinomas occurred more frequently, as often observed in aging rats (Thoolen et al. 2010). These tumors appeared to be incidental and not compound-related, as their frequency of occurrence was not dependent on dose. Hepatocellular adenomas and carcinomas were considered select neoplasms (Table 9), based on increased incidence above controls for total animals, albeit non-dose

Table 9. Study 4 – Two-year feeding study of glyphosate in rats (Feinchemie Schwebda 1996).

Study owner:	Feinchemie Schwebda (1996)			
Reliability/Justification:	1 Study performed according to GLP and OECD guideline requirements, with no deviations.			
Substance:	Glyphosate (96.0–96.8% pure)			
Species/Strain:	Rat/Wistar, groups of 50 ♂ and 50 ♀			
Administration route:	Diet			
Concentration:	0, 100, 1000, 10 000 ppm diet (♂ about 0, 6.3, 59.4, 595 mg/kg bw/day; ♀ about 0, 8.6, 88.5, 886 mg/kg bw/day)			
Duration:	2 years			
Findings:	10 000 ppm diet: ≥ NOAEL (♂+♀) Only mild effects on clinical chemistry (liver enzymes), without histopathological changes.			
Select neoplasms:	Hepatocellular adenoma, hepatocellular carcinoma			
Tumor		Dose (mg/kg bw/day)		
Males	0	7.4	73.9	741
Findings for dead and moribund sacrificed animals				
Hepatocellular adenoma – B	9/30 (30%)	9/30 (30%)	6/32 (19%)	6/21 (29%)
Hepatocellular carcinoma – M	12/30 (40%)	12/30 (40%)	9/32 (28%)	5/21 (24%)
Findings for animals sacrificed at termination				
Hepatocellular adenoma – B	15/20 (75%)	13/20 (65%)	4/16 (25%)	15/20 (75%)
Hepatocellular carcinoma – M	9/20 (45%)	16/20 (80%)	9/16 (56%)	19/29 (66%)
		Dose (mg/kg bw/day)		
Females	0	7.4	73.9	741
Findings for dead and moribund sacrificed animals				
Hepatocellular adenoma – B	2/26 (8%)	8/23 (3%)	3/17 (18%)	5/29 (17%)
Hepatocellular carcinoma – M	4/26 (15%)	4/23 (17%)	2/17 (12%)	5/29 (17%)
Findings for animals sacrificed at termination				
Hepatocellular adenoma – B	16/24 (67%)	10/25 (40%)	16/32 (50%)	8/21 (38%)
Hepatocellular carcinoma – M	6/24 (25%)	11/25 (44%)	12/32 (38%)	4/21 (19%)

B benign, *M* malignant

Case 3:16-md-02741-VC Document 654-2 Filed 10/28/17 Page 32 of 138

responsive, for adenoma in mid-dose females, carcinoma in low- and high-dose males, and carcinoma in low- and mid-dose females. These liver neoplasms are considered in the weight of evidence evaluation (Tables 20 and 21).

The study report concluded that glyphosate technical acid was not carcinogenic in Wistar rats following continuous dietary exposure of up to 595 and 886 mg/kg bw/day in males and females, respectively, for 24 months, which is consistent with evaluations by the European Authorities (EC 2002, Germany Rapporteur Member State 2015b).

Study 5 (Excel 1997)

A 2-year feeding study in the Sprague Dawley rats (Excel 1997) featured dietary concentrations of 0, 3000, 15 000, and 25 000 ppm glyphosate technical acid. Groups of 50 rats per sex were fed for 24 months, and mean dose levels of 0, 150, 780 and 1290 mg/kg bw/day (males) and 0, 210, 1060 and 1740 mg/kg bw/day (females) were achieved (Table 10).

In addition, 20 rats/sex/group were included for interim sacrifice at week-52, to study non-neoplastic histopathological changes with a different high-dose level of 30 000 ppm. The dietary doses correspond to 180, 920 and 1920 mg/kg bw/day (males) and 240, 1130 and 2540 mg/kg bw/day (females), for 3000, 15 000 and 30 000 ppm, respectively. Thus, a limit dose above 1000 mg/kg bw/day was achieved.

The study report notes that glyphosate technical acid was not carcinogenic in Sprague Dawley rats following continuous dietary exposure to up to 1290 mg/kg bw/day, and 1740 mg/kg bw/day for males and females, respectively, for 24 months. However, this study was rated Klimisch 3 for reliability (Germany Rapporteur Member State 2015b), and therefore, is considered unreliable for carcinogenicity evaluation based on lower than expected background tumor incidences (see data Supplementary Study 5 to be found online at http://informahealthcare.com/doi/abs/10.3109/10408444.2014.1003423). In addition, the test substance was not adequately characterized, and several deviations from the OECD Test Guideline 453 were noted.

Study 6 (Arysta Life Sciences 1997b)

A combined chronic toxicity/carcinogenicity study in Sprague Dawley rats (Arysta Life Sciences 1997b) was conducted between December 1994 and December 1996. The rats were fed 0, 3000, 10 000, and 30 000 ppm glyphosate for two years (equivalent to 0, 104, 354 and 1127 mg/kg bw/day for males and 0, 115, 393 and 1247 mg/kg bw/day for females (Table 11). Thus, a limit dose was achieved, and the MTD was noted at the high dose in males and females with decreased body weight, increased cecum weight, distention of the cecum, loose stool and skin lesions. In addition, 30 rats/sex/group were included for interim sacrifice at 26, 52 and 78 weeks, to study non-neoplastic histopathological changes. Observations covered clinical signs, body weight, food consumption, hematology, clinical chemistry, and urinalysis, as well as organ weights, necropsy, and histopathological examination. This study was rated Klimisch 1 for reliability.

Non-statistically significant increases versus controls ($p < 0.05$) were noted for pituitary adenomas, skin keratoacanthoma in high-dose males, and mammary gland fibroadenoma in low and mid-dose females (Table 11). These neoplasms were considered for the weight of evidence evaluation (Tables 20 and 21), and the full tumor summary data are available online (see data Supplementary Study 6 to be found online at http://informahealthcare.com/doi/abs/10.3109/10408444.2014.1003423). As mentioned under Study 1, pituitary and mammary tumors are common spontaneous neoplasms in aging rats (Johnson and Gad 2008), and skin keratoacanthoma is noted as one of the most common spontaneous benign neoplasms in male Sprague Dawley rats (Chandra et al. 1992). The study report concluded that glyphosate was not carcinogenic in Sprague Dawley rats following continuous dietary exposure to up to 30 000 ppm for 24 months, corresponding to 1127 mg/kg bw/day and 1247 mg/kg bw/day for males and females, respectively, which is consistent with the recent evaluation in Europe under the Annex I Renewal of glyphosate (Germany Rapporteur Member State 2015b).

Table 10. Study 5 – Two-year feeding study of glyphosate in rats (Excel 1997).

Study owner:	Excel (1997)
Reliability/Justification:	3 Test substance not characterized and other deviations from OECD 453, lower than expected background tumor incidence
Substance:	Glyphosate (no purity reported)
Species/Strain:	Rat/Sprague-Dawley, groups of 50 ♂ and 50 ♀, additional groups of 20 rats per sex and group were included for interim sacrifice after 52 weeks
Administration route:	Diet
Concentration:	2-year group: 0, 3000, 15 000, 25 000 ppm diet (♂ about 0, 150, 780, 1290 mg/kg bw/day; ♀ about 0, 210, 1060, 1740 mg/kg bw/day) 1-year group: 0, 3000, 15 000, 30 000 ppm diet (♂ about 0, 180, 920, 1920 mg/kg bw/day; ♀ about 0, 240, 1130, 2540 mg/kg bw/day)
Duration:	2 years
Findings:	≥ 25 000 ppm diet: NOAEL (♂+♀) Only mild toxic effects, such as clinical chemistry of questionable relevance in aged rats, without correlating histopathological organ changes.
Select neoplasms:	No neoplasms from this study were identified for further consideration. Low background tumor incidence indicates low study reliability with no relevant increases in the incidence of tumors.

Males	Dose (mg/kg bw/day)			
	0	150	740.6	1290
Mortality	16/50 (32%)	17/50 (34%)	18/50 (36%)	23/50 (46%)
Females	Dose (mg/kg bw/day)			
	0	210	1060	1740
Mortality	19/50 (38%)	20/50 (40%)	20/50 (40%)	25/50 (50%)

Table 11. Study 6 – Two-year feeding study of glyphosate in rats (Arysta Life Sciences 1997b).

Study owner:	Arysta Life Sciences (1997b)			
Reliability/Justification:	1 Study performed according to GLP and OECD guideline requirements. with no deviations.			
Substance:	Glyphosate (94.6–97.6% pure)			
Species/Strain:	Rat/Sprague-Dawley, groups of 50 ♂ and 50 ♀; satellite groups of 30 ♂ and 30 ♀for interim investigations			
Administration route:	Diet			
Concentration:	0, 3000, 10 000, 30 000 ppm diet (♂ about 0, 104, 354, 1127 mg/kg bw/day; ♀ about 0, 115, 393, 1247 mg/kg bw/day)			
Duration:	2 years			
Findings:	3000 ppm diet: NOAEL (♂+♀)			
	10 000 ppm diet: cecum weight↑, distension of cecum, loose stool, follicular hyperkeratosis and/or folliculitis/follicular abscess of the skin, body weight ↓			
Select neoplasms:	Pituitary adenoma, skin keratoacanthoma (males), mammary gland fibroadenoma (females)			
Tumor		Dose (mg/kg bw/day)		
Males	0	104	354	1127
Findings for dead and moribund sacrificed animals (Table 25–10)				
Pituitary anterior adenoma – B	22/32 (69%)	21/30 (70%)	*14/32 (44%)	18/21 (86%)
Skin keratoacanthoma – B	2/32 (6%)	1/30 (3%)	0/32	1/21 (5%)
Findings for animals sacrificed at termination (after 104 weeks, Table 25–8)				
Lung adenoma – B	0/18	2/20 (10%)	1/18 (6%)	3/29 (10%)
Pituitary anterior adenoma – B	13/18 (72%)	14/20 (70%)	13/18 (72%)	21/29 (72%)
Pituitary adenoma in intermediate part – B	0/18	1/20 (5%)	0/18	0/29 (0%)
Skin keratoacanthoma – B	1/18 (6%)	2/20 (10%)	0/18	6/29 (21%)
Tumor		Dose (mg/kg bw/day)		
Females	0	115	393	1247
Findings for dead and moribund sacrificed animals				
Pituitary anterior adenoma – B	34/35 (97%)	29/31 (94%)	28/33 (82%)	31/36 (86%)
Thyroid follicular adenoma – B	0/35	2/31 (6%)	0/32	0/36
Mammary gland fibroadenoma – B	13/35 (37%)	14/31 (45%)	12/34 (35%)	20/36 (56%)
Findings for animals sacrificed at termination				
Pituitary anterior adenoma – B	12/15 (80%)	19/19 (100%)	12/16 (75%)	13/14 (93%)
Mammary gland fibroadenoma – B	10/15 (67%)	13/19 (68%)	12/16 (75%)	10/14 (71%)

B benign. *M* malignant
*Statistically lower than controls ($p < 0.05$).

Study 7 (Syngenta 2001)

The same rat model that was used in the previously discussed 12-month chronic rat study (Syngenta 1996b) was also employed in a 2-year feeding study (Syngenta 2001). A group of 52 male and 52 female Wistar rats received 0, 2000, 6000 or 20 000 ppm via feed (Table 12). The mean achieved dose levels were 0, 121, 361 and 1214 mg/kg bw/day for males, and 0, 145, 437 and 1498 mg/kg bw/day for females. Thus, a limit dose was achieved. In addition, three satellite groups with 12 rats per sex each were included for interim sacrifice after 12 months of treatment, to investigate potential non-neoplastic histopathological changes. Observations covered clinical signs, body weight, food consumption, hematology, clinical chemistry, and urinalysis, as well as organ weights, necropsy, and histopathological examination. This study was rated Klimisch 1 for reliability.

Treatment-related findings in this study were found in the liver and kidney, and were confined to animals (predominantly males) fed 20 000 ppm glyphosate acid. There were a number of changes in males and females fed 20 000 ppm glyphosate acid, notably renal papillary necrosis, prostatitis, periodontal inflammation, urinary acidosis, and hematuria, which may be attributed to the acidity of the test substance. Slight increases in proliferative cholangitis and hepatitis were noted in males at 20 000 ppm. Despite the findings at 20 000 ppm, survival was better in males fed 20 000 ppm than in the controls and lower dose groups. This improved survival was associated with a decreased severity of renal glomerular nephropathy and a 5% reduction in body weight (see data Supplementary Study 7 to be found online at http://informahealthcare.com/doi/abs/10.3109/10408444.2014.1003423, for neoplastic and non-neoplastic findings).

A small increase in the incidence of hepatocellular adenoma was observed in males fed 20 000 ppm glyphosate acid. While not statistically significant using the Fisher's exact test, the difference was statistically significant for total male rats using the Peto Test for trend. However, there was no evidence of pre-neoplastic foci, no evidence of progression to adenocarcinomas, and no dose-response. In addition, the incidence was within the laboratory's historical control range for tumors of this type in the liver (Table 12). Therefore, the increased incidence was considered not to be related to treatment, yet these were considered select neoplasms (Table 12) and evaluated in context of the complete data set (Tables 20 and 21).

The study report concluded that glyphosate acid was not carcinogenic in the Wistar rats following continuous dietary exposure to up to 20 000 ppm for 24 months, at 1214 and 1498 mg/kg bw/day in males and females, respectively, which is consistent with the WHO/FAO review (WHO/FAO 2004a) and the recent evaluation in Europe under the Annex I Renewal of glyphosate (Germany Rapporteur Member State 2015b).

Study 8 (Nufarm 2009b)

The most recent study in this series of regulatory studies investigating the potential carcinogenicity of glyphosate in rats was conducted from September 2005 through March 2008 (Nufarm 2009b). The study was conducted by feeding dietary concentrations of 0, 1500, 5000 and 15 000 ppm glyphosate to groups of 51 Wistar rats per sex. To ensure that a received limit dose of 1000 mg/kg bw/day overall was achieved, the highest dose level was progressively increased to 24 000 ppm.

 Case 3:16-md-02741-VC Document 654-2 Filed 10/28/17 Page 34 of 138

Table 12. Study 7 – Two-year feeding study of glyphosate in rats (Syngenta 2001).

Study owner:	Syngenta (2001)			
Reliability/Justification	1 Study performed according to GLP and OECD guideline requirements, with no deviations.			
Substance:	Glyphosate (97.6% pure)			
Species/Strain	Rat/Wistar Alpk: AP$_f$SD, groups of 52 ♂ and 52 ♀ (additional 12 animals per sex and dose for 1-year interim sacrifice)			
Administration route:	Diet			
Concentration:	0, 2000, 6000, 20 000 ppm diet (♂ about 0, 121, 361, 1214 mg/kg bw/day; ♀ about 0, 145, 437, 1498 mg/kg bw/day)			
Duration:	2 years			
Findings:	6000 ppm diet: NOAEL (♂+♀)			
	20 000 ppm diet: Kidney and liver findings. Increased survival due to reduction in CPN, prostatitis, periodontal inflammation			
Select neoplasms:	Hepatocellular adenoma (males), not a statistically significant increase for the high dose using the Fisher's exact test, but statistically significant using Peto trend analysis			
		Dose (mg/kg bw/day)		
Males	0	121	361	1214
Liver				
Hepatocyte fat vacuolation	6	7	11	11
Hepatitis	3	4	2	5
Kidney				
		Dose (mg/kg bw/day)		
Females	0	145	437	1498
Liver				
Hepatocyte fat vacuolation	7	5	6	6
Hepatitis	6	5	4	4
Tumors:		Dose (mg/kg bw/day)		
Males	0	121	361	1214
Findings for dead and moribund sacrificed animals				
*Hepatocellular adenoma – B	0/37	2/36 (6%)	0/35	3/26 (12%)
Hepatocellular carcinoma – M	0/37	0/36	0/35	0/26
Findings for animals sacrificed at termination				
*Hepatocellular adenoma – B	0/16	0/17	0/18	2/26 (8%)
Hepatocellular carcinoma – M	0/16	0/17	0/18	0/26

B benign, *M* malignant
*Historical Control Range: 0–11.5% total males with hepatocellular adenoma, 26 studies, 1984–2003

Mean dose levels of 86/105, 285/349, and 1077/1382 mg glyphosate/kg bw/day (males/females) were achieved (Table 13). This study was rated Klimisch 1 for reliability.

Non-neoplastic findings included transient liver enzyme activity for mid-dose males and high-dose males and females, and equivocal nephrocalcinosis depositions at the high-dose. Histopathology noted a statistically significant increase in adipose infiltration of the bone marrow in high-dose males compared to controls, suggestive of myeloid hypoplasia, which may be considered a stress response (Everds et al. 2013).

Skin keratoacanthoma in males and mammary gland adenocarcinoma in females (Table 13) were considered for evaluation in the context of the weight of evidence for rat tumor incidence (Tables 20 and 21), wherein dose-

Table 13. Study 8 – Two-year feeding study of glyphosate in rats (Nufarm 2009b).

Study owner:	Nufarm (2009a)			
Reliability/Justification:	1 Study performed according to GLP and OECD guideline requirements, with no deviations			
Substance:	Glyphosate (95.7% pure)			
Species/Strain:	Rat/Wistar, groups of 51 ♂ and 51 ♀			
Administration route:	Diet			
Concentration:	0, 3000, 10 000, 15 000 ppm diet, the top dose was progressively increased to reach 24 000 ppm diet by Week-40 (♂ about 0, 84, 285, 1077 mg/kg bw/day; ♀ about 0, 105, 349, 1382 mg/kg bw/day)			
Duration:	2 years			
Findings:	≥ 1077/1382 mg/kg bw/day: NOAEL (♂/♀)			
	Transient liver enzyme activity for mid-dose males and high-dose males and females; equivocal nephrocalcinosis depositions at the high-dose males and females; increased adipose infiltration of the bone marrow in high-dose males			
Select neoplasms:	Skin keratoacanthoma (males), mammary gland adenocarcinoma			
Tumor			Dose (mg/kg bw/day)	
Males	0	84	285	1077
Findings for all animals				
Skin keratoacanthoma – B	2/51 (4%)	3/51 (6%)	0/51	6/51 (12%)
			Dose (mg/kg bw/day)	
Females	0	105	349	1382
Findings for all animals				
Mammary gland adenocarcinoma – M	2/51 (4%)	3/51 (6%)	1/51 (2%)	6/51 (12%)

B benign, *M* malignant

responses were not evident. Tumor incidence summary data have been tabulated (see data Supplementary Study 8 to be found online at http://informahealthcare.com/doi/abs/10.3109/10408444.2014.1003423). Microscopic evaluation of tissues did not reveal any indications of neoplastic lesions caused by glyphosate treatment. The study report concluded that glyphosate acid was not carcinogenic in Wistar rats following continuous dietary exposure to up to 24 000 ppm for 24 months, at 1077 and 1382 mg/kg bw/day in males and females, respectively, which is consistent with the recent evaluation in Europe under the Annex I Renewal of glyphosate (Germany Rapporteur Member State 2015b).

Study 9 Publication (Chruscielska et al. 2000a)

A two-year combined chronic toxicity and carcinogenicity study in Wistar rats was published by academic researchers from Warsaw, Poland. The study was conducted as a drinking-water study in Wistar-RIZ rats according to OECD TG 453. The test material was a 13.85% aqueous formulation of glyphosate as its ammonium salt (equivalent to 12.6% glyphosate acid). However, the ammonium salt of glyphosate tested is not commercially available, and the concentration of active ingredient suggests that a glyphosate-formulated product was tested; this is supported by a concurrent genotoxicity publication by the same lead author (Chruscielska et al. 2000b), previously reviewed by Kier and Kirkland (Kier and Kirkland 2013), in which a glyphosate formulation, Perzocyd, was tested. Deficiencies noted with respect to OECD TG 453 include insufficient dosing to elicit toxic effects, inadequate test material characterization, no reporting of water/feed consumption, body weights and diet composition, and no individual animal data. Although the manuscript reporting deficiencies may have been included in the study, they were not reported in the manuscript, and could warrant a Klimisch reliability score of 4 (not assignable), but the low doses employed in this study justify a Klimisch reliability score of 3.

The test material was administered in water at glyphosate salt concentrations of 0, 300, 900, and 2700 mg/L. Each dose group consisted of 85 animals per sex. Ten animals per sex and dose were sacrificed after 6, 12, and 18 months of exposure, for evaluation of general toxicity. The remaining 55 animals per sex and dose were scheduled for sacrifice after 2 years of exposure.

Water consumption was claimed to have been measured, but these data have not been reported. To estimate the glyphosate doses received via drinking water, the assumed default water consumptions were 50 and 57 mL/kg bw/day by male and female rats, respectively (Gold et al. 1984). Using these standard figures and the glyphosate content of the tested formulation (12.6%), daily doses are estimated at 0, 1.9, 5.7, and 17 mg of glyphosate/kg bw/day for males and 0, 2.2, 6.5, and 19 mg of glyphosate/kg bw/day for females. As this study appears to have tested a formulated product, data were not included in the weight of evidence review (Tables 20 and 21), but given the very low glyphosate doses and reported low tumor incidence, these were of no consequence to the overall data review.

Exposure to glyphosate ammonium salt had no effect on body weight, appearance and behavior, and hematological parameters, which is consistent with glyphosate chronic toxicity data regulatory reviews. Even though there seems to be a trend towards higher 2-year mortality in treated females (Table 14), this difference had no statistical significance according to the authors. There were sporadic alterations of clinical-chemical and urinalysis parameters, but not in a consistent fashion over time and without dose-dependence. These alterations were not interpreted as treatment-related. There was no effect of glyphosate on the incidence of neoplastic lesions (Table 14). Thus, the NOAEL for chronic toxicity and carcinogenicity in this study was greater than or equal to 17 and 19 mg glyphosate/kg bw/day, in males and females, respectively.

Due to the lack of systemic effects in the highest dose group, the MTD was not reached by this study. Judging from other rat studies reviewed here, the MTD is likely to be greater than 1000 mg/kg bw/day. Thus, the top glyphosate dose of an estimated 19 mg/kg bw/day in this study is too low to satisfy regulatory validity criteria for a carcinogenicity study.

Mouse carcinogenicity

There are a total of five carcinogenicity studies with glyphosate in mice, that have been submitted to support glyphosate Annex I renewal in the European Union. All but the oldest study (Study 10) were considered reliable without restriction, and were performed under conditions of GLP following OECD TGs. Most studies were conducted in the CD-1 strain. Each study was sponsored by a different manufacturer. In each case, technical grade glyphosate was administered via diet for at least 18 months. Select neoplasms, mostly lymphoreticular, liver and lung, are summarized for all mouse chronic studies in Tables 22 and 23. These neoplasms are widely recognized as occurring spontaneously in aging mice (Gad et al. 2008, Son and Gopinath 2004). Lymphomas have been recognized for many years as one of the most common, if not the most common category of spontaneous neoplastic lesions in aging mice (Brayton et al. 2012, Gad et al. 2008, Son and Gopinath 2004). The subclassification of malignant lymphomas is not a typical diagnostic feature in rodent studies, likely due to either expense and/or feasibility. It is, however, important to recognize that lymphomas are not a single type of neoplasm, rather they are a grouping of different neoplasms arising from different pathogeneses, and should be considered as different diseases (Bradley et al. 2012). As is the case for NHL in humans, these different immune system neoplasms are clustered together based on manifestation in lymphocytes, despite their very different etiologies; for example, the most common subset of NHL lymphomas clustered together as "diffuse large B cell lymphomas", have for many years been considered multiple clinical-pathologic entities (Armitage 1997), and therefore may be considered attributable to different modes of action. Chronic endpoints and NOAEL values are captured in each study summary table; however, the following study reviews focus on carcinogenicity.

Study 10 (Monsanto 1983)

The first chronic-carcinogenicity mouse study with glyphosate was conducted between March 1980 and March 1982 (Monsanto 1983), prior to the institution of GLP (Table 15). The study design was essentially in compliance with OECD TG 451 for carcinogenicity studies, adopted in 1981, when

 Case 3:16-md-02741-VC Document 654-2 Filed 10/28/17 Page 36 of 138

Table 14. Publication, Study 9 – Two-year drinking water study in rats with 13.85% glyphosate ammonium salt (Chruscielska et al. 2000a).

Authors:	Chruscielska et al. (2000a)
Reliability/Justification:	3 Study not performed according to GLP, but according to OECD TG 453, with the following deficiencies: Reporting deficits (water and feed consumption, body weights, diet composition, individual animal data, substance composition, purity, and stability) Highest dose did not elicit toxicity.
Substance:	Ammonium salt of glyphosate, 13.85% solution
Species/Strain:	Rat/Wistar -RIZ outbred, 85 ♂ and 85 ♀ per dose group. 10 ♂ and 10 ♀each were sacrificed after 6, 12, and 18 months of exposure.
Administration route:	Drinking water
Concentration:	0, 300, 900, and 2700 mg/L Estimated glyphosate intake: ♂: 0, 1.9, 5.7, and 17 mg/kg bw/day. ♀: 0, 2.2, 6.5, and 19 mg/kg bw/day, based on assumed water consumptions of 50/57 mL/kg bw/day (♂/♀), (Gold, et al. 1984)
Duration:	2 years
Findings:	17/19 mg glyphosate/kg bw/day: NOAEL (♂/♀) No treatment-related effects
Tumors reported for 85 rats/sex/dose:	No increase in the incidence of tumors attributable to glyphosate administration

	Estimated dose (mg/kg bw/day)							
	0		1.9/2.2		5.7/6.5		17/19	
	♂	♀	♂	♀	♂	♀	♂	♀
Two-year mortality	42%	38%	42%	45%	54%	53%	44%	60%
Lungs								
Lymphoma	2	–	2	–	1	–	3	1
Histiocytoma	–	–	–	–	–	–	–	1
Adenocarcinoma	1	–	–	–	–	–	–	–
Histiocytoma, malignant	–	1	–	–	1	–	–	–
Spleen, leukemia	0	–	2	–	0	–	1	–
Kidneys, Fibrous histiocytoma	–	–	–	–	–	–	1	–
Pituitary gland								
Adenoma	4	10	4	6	2	8	0	3
Adenoma, malignant (assumed to be carcinoma)	0	1	0	3	1	2	1	5
Carcinoma	0	–	0	–	1	–	0	–
Thyroid								
Adenoma	1	1	1	2	0	0	3	3
Carcinoma	0	–	1	–	0	–	0	–
Uterus, cervix carcinoma	–	0	–	0	–	0	–	1
Uterus, body, histiocytoma	–	3	–	1	–	0	–	1
Mammary gland								
Fibroma	–	0	–	0	–	0	–	0
Fibroadenoma	–	3	–	2	–	3	–	3
Adrenal medulla, adenoma	1	2	2	2	1	2	0	2
Thymus, lymphoma	0		0		0		1	
Testis, Leydigoma	–		3		6		1	
Subcutaneous tissue								
Fibroma	0		1		1		3	
Lipoma	–	–	–	–	–	–	–	1
Cystadenoma	–	1	–	–	–	–	–	–
Lymph nodes								
Lymphoma	0		0		0		1	
Lymphoma, malignant	–	1	–	–	–	–	–	–
Skin, carcinoma	2	–	–	–	–	–	–	–
Prostate, adenoma	1	–	–	–	–	–	–	–

the study was already ongoing. Groups of 50 male and female CD-1 mice received glyphosate at dietary levels of 1000, 5000, and 30 000 ppm, over a period of nearly two years. The mean achieved doses were 157/190, 814/955, and 4841/5874 mg/kg bw/day in males and females, respectively, exceeding the limit dose. Based on this study predating both GLP and OECD TG 451, a reliability score of Klimisch 2 has been assigned.

In addition to post-mortem pathological examinations after terminal sacrifice, hematological investigations were performed on 10 mice per sex and dose at months 12 and 18, and on 12 male animals/group, as well as all surviving females at scheduled termination.

Two non-neoplastic histological changes affecting the liver and urinary bladder were assumed to be treatment-related. There was a higher incidence of centrilobular hepatocyte hypertrophy in high-dose males, and a more frequent occurrence of slight-to-mild bladder epithelial hyperplasia in the mid and high dose; however, a clear dose-response was lacking. Tumor incidences, which did not significantly increase with dose, were mostly bronchiolar-alveolar, hepatocellular, or lymphoreticular, all of which are commonly noted spontaneously occurring tumors in aging mice (Table 15). Lymphoreticular tumors combined for males and females totaled 7, 12, 10 and 12 for control, low, mid- and high-dose groups respectively, and were not considered as being related to test substance.

A more frequent occurrence of slight-to-mild bladder epithelial hyperplasia was observed in the mid and high-dose groups; however, clear dose-response was lacking (Table 15) and no urinary bladder neoplasms were noted at these doses (see data Supplementary Study 10 to be found online at http://

Case 3:16-md-02741-VC Document 654-2 Filed 10/26/17 Page 37 of 138

Table 15. Study 10 – Two-year feeding study with glyphosate in mice (Monsanto 1983).

Study owner:	Monsanto (1983)			
Reliability/Justification	2 Study was performed prior to institution of GLP and OECD guideline requirements			
Substance:	Glyphosate (99.7% pure)			
Species/Strain:	Mouse/CD-1, groups of 50 ♂ and 50 ♀			
Administration route:	Diet			
Concentration:	0, 1000, 5000, 10 000 ppm diet (♂ about 0, 157, 814, 4841 mg/kg bw/day; ♀ about 0, 190, 955, 5874 mg/kg bw/day)			
Duration:	24 months			
Findings:	1000 ppm diet: NOAEL (♂ + ♀) 5000 ppm diet: body weight ↓, histological changes in liver and urinary bladder (slight to mild epithelial hyperplasia in males at mid and high doses)			
Select neoplasms:	Lymphoreticular neoplasms, bronchiolar-alveolar adenocarcinoma			
		Dose (mg/kg bw/day)		
Males	0	157	814	4841
Lymphoreticular system				
Lymphoblastic lymphosarcoma with leukemia – M	1/48 (2%)	4/49 (8%)	3/50 (6%)	2/49 (4%)
Lymphoblastic lymphosarcoma without leukemia – M	0/48	1/49 (2%)	0/50 (0%)	0/49
Composite lymphosarcoma – M	1/48 (2%)	0/49	1/50 (2%)	0/49
Histiocytic sarcoma – M	0/48	1/49 (2%)	0/50	0/49
Total lymphoreticular neoplasms[#]	2/48 (4%)	6/49 (12%)	4/50 (8%)	2/49 (4%)
		Dose (mg/kg bw/day)		
Females	0	190	955	5873
Lymphoreticular system				
Lymphoblastic lymphosarcoma with leukemia – M	1/50 (2%)	4/48 (8%)	5/49 (10%)	1/49 (2%)
Lymphoblastic lymphosarcoma without leukemia – M	0/50 (0%)	1/48 (2%)	0/49 (0%)	3/49 (6%)
Composite lymphosarcoma – M	4/50 (8%)	1/48 (2%)	1/49 (2%)	6/49 (12%)
Histiocytic sarcoma – M	0/50 (0%)	0/48 (0%)	0/49 (0%)	0/49 (0%)
[#] Total lymphoreticular neoplasms	5/50 (10%)	6/48 (13%)	6/49 (12%)	10/49 (20%)

[#]Sum of lymphoblastic lymphosarcoma, composite lymphosarcoma, and histiocytic sarcoma.
M malignant

informahealthcare.com/doi/abs/10.3109/10408444.2014.1003423). Benign renal tubule adenomas were noted in mid- and high-dose males at incidences of 1/50 and 3/50 respectively. These neoplasms were not observed in females, lacked statistical significance, and were considered spontaneous and unrelated to glyphosate administration by the study pathologists; this neoplasm, while not seen in the concurrent control group, had previously been noted in control male CD-1 mice of comparable age by the author of the study. As an additional measure of diligence, a Pathology Working Group was convened, and it concluded that the absence of any pre-neoplastic kidney lesion in all male animals provided sufficient evidence that this finding was spurious and not related to glyphosate administration. This is reflected in the US EPA review of glyphosate (US EPA 1993). This neoplasm was not observed in the other four mouse carcinogenicity studies discussed.

The author of the study also reported a trend towards a non-statistically significant increased occurrence of lymphoreticular neoplasia in treated female mice (Table 15). However, these consisted of three different categories of lymphoreticular neoplasms. Regulatory reviews confirmed that there is no apparent dose-dependence for these endpoints (EC 2002, US EPA 1993, WHO/FAO 2004a). Summary tables of incidence of neoplastic findings are available (see data Supplementary Study 10 to be found online at http://informahealthcare.com/doi/abs/10.3109/10408444.2014.1003423).

Glyphosate was reported as not carcinogenic in CD-1 mice up to doses well in excess of the limit dose for carcinogenicity testing, which is consistent with evaluations by the US EPA (US EPA 1993), European Commission (EC 2002), recent EU Annex I Renewal evaluation by the Rapporteur (Germany Rapporteur Member State 2015b), and WHO/FAO (WHO/FAO 2004a).

Study 11 (Cheminova 1993b)

Another carcinogenicity bioassay in mice was conducted between December 1989 and December 1991 (Table 16) (Cheminova 1993b). In this assay, 50 male and 50 female CD-1 mice per dose group received glyphosate via their diet over a period of approximately two years. This treatment period is 6 months longer than the 18 months stipulated for mice by OECD TG 451 (1981 version). The dietary levels were adjusted regularly to achieve constant dose levels of 0, 100, 300 and 1000 mg/kg bw/day, achieving the limit dose. This study was rated Klimisch 1 for reliability.

Slight non-statistically significant increases in bronchiolar-alveolar adenomas were noted for all male dose groups above controls in a non-dose-responsive manner. Bronchiolar-alveolar neoplasms are evaluated in the context of the full data set (Tables 22 and 23), demonstrating a lack of dose-response across doses ranging from approximately 15 mg/kg bw/day to 5000 mg/kg bw/day. Although the number of pituitary adenomas were low and considered incidental, they were conservatively included in the select neoplasms, based on being slightly higher in high dose females than concurrent controls (Table 16). The data summary of all histological findings, including tumor incidence, is available (see data Supplementary Study 11 to be found online at http://informahealthcare.com/doi/abs/10.3109/10408444.2014.1003423).

There were no statistically significant increases in the occurrence of any tumor type in this study. The observed variations did not show a dose relationship, and were within the range of historical control data. Glyphosate was determined to be not carcinogenic to CD-1 mice at up to 1000 mg/kg bw/day, which is consistent with evaluations by the European Commission (EC 2002) and WHO/FAO (WHO/FAO 2004a).

 Case 3:16-md-02741-VC Document 654-2 Filed 10/28/17 Page 38 of 138

Table 16. Study 11 – Two-year feeding study with glyphosate in mice (Cheminova 1993b).

Study owner:	Cheminova (1993b)
Reliability/Justification:	1 Study performed according to GLP and OECD guideline requirements
Substance:	Glyphosate (98.6% pure)
Species/Strain:	Mouse/CD-1, groups of 50 ♂ and 50 ♀
Administration route:	Diet
Concentration:	♂+♀: 0, 100, 300, 1000 mg/kg bw/day (regular adjustment of dietary concentration)
Duration:	24 months
Findings:	≥ 1000 mg/kg bw/day: NOAEL (♂+♀) no treatment-related effects
Select neoplasms:	Bronchiolar-alveolar adenoma, bronchiolar-alveolar carcinoma, pituitary adenoma (females)

	Dose (mg/kg bw/day)			
Males	0	10	300	1000
Bronchiolar-alveolar adenoma – B	9/50 (18%)	15/50 (30%)	11/50 (22%)	13/50 (26%)
Bronchiolar-alveolar carcinoma – M	10/50 (20%)	7/50 (14%)	8/50 (16%)	9/50 (18%)
	Dose (mg/kg bw/day)			
Females	0	100	300	1000
Bronchiolar-alveolar adenoma – B	7/50 (14%)	3/50 (6%)	3/50 (6%)	6/50 (12%)
Bronchiolar-alveolar carcinoma – M	3/50 (6%)	2/50 (4%)	1/50 (2%)	5/50 (10%)
Pituitary adenoma – B	1/41 (2%)	0/32	0/23	3/43 (6%)

B benign, *M* malignant

Study 12 (Arysta Life Sciences 1997a)

An 18-month feeding study in ICR-CD-1 mice, conducted between February 1995 and September 1996, investigated higher doses by admixing 1600, 8000, or 40 000 ppm glyphosate into the diet fed to groups of 50 male and 50 female mice per dose (Arysta Life Sciences 1997a). The calculated test substance intake was 165/153, 838/787, and 4348/4116 mg/kg bw/day (males/females, Table 17), exceeding the limit dose. This study was rated Klimisch 1 for reliability.

Histopathological examinations did not show statistically significant increases for any type of neoplastic lesion in all treatment groups of both sexes (see data Supplementary Study 12 to be found online at http://informahealthcare.com/doi/abs/10.3109/10408444.2014.1003423). Select neoplasms evaluated across the data set with some non-statistically significant increases above concurrent controls included lymphoma and lung tumors, all of which lacked a clear dose-response. Glyphosate was considered not carcinogenic in CD-1 mice up to doses well in excess of the limit dose for carcinogenicity testing, which is consistent with the recent evaluation in Europe under the Annex I Renewal of glyphosate (Germany Rapporteur Member State 2015b).

Study 13 (Feinchemie Schwebda 2001)

An 18-month feeding study in Swiss albino mice (Feinchemie Schwebda 2001), conducted between December 1997 and June 1999, featured treatment groups, each with 50 animals per sex, receiving 100, 1000, and 10 000 ppm technical grade glyphosate

Table 17. Study 12 – Two-year feeding study with glyphosate in mice (Arysta Life Sciences 1997a).

Study owner:	Arysta Life Sciences (1997b)
Reliability/ Justification:	1 Study performed according to GLP and OECD guideline requirements, with no deviations.
Substance:	Glyphosate (94.6–97.6% pure)
Species/Strain	Mouse/CD-1, groups of 50 ♂ and 50 ♀
Administration route:	Diet
Concentration:	0, 1600, 8000, or 40 000 ppm diet (♂ about 0, 165, 838, 4348 mg/kg bw/day; ♀ about 0, 153, 787, 4116 mg/kg bw/day)
Duration:	18 months
Findings:	8000/1600 ppm diet: NOAEL (♂/♀) 8000 ppm diet (♀): retarded growth 40 000 ppm diet: pale-colored skin ♂, loose stool, retarded growth, reduced food consumption and food efficiency, cecum distension and increased absolute and relative cecum weight, without histopathological findings of increased incidence of anal prolapse, consistent with histopathological erosion/ulcer of the anus
Select neoplasms:	Lung adenoma, lung adenocarcinoma, lymphoma

	Dose (mg/kg bw/day)			
Males	0	165	838	4348
Lung adenoma – B	8/50 (16%)	14/50 (28%)	13/50 (26%)	11/50 (11%)
Lung adenocarcinoma – M	1/50 (2%)	1/50 (2%)	6/50 (12%)	4/50 (8%)
Lymphoma – M	2/50 (4%)	2/50 (4%)	0/50	6/50 (12%)
	Dose (mg/kg bw/day)			
Females	0	153	787	4116
Lung adenoma – B	8/50 (16%)	5/50 (10%)	12/50 (24%)	5/50 (10%)
Lung adenocarcinoma – M	1/50 (2%)	2/50 (4%)	3/50 (6%)	1/50 (2%)
Lymphoma – M	6/50 (12%)	4/50 (8%)	8/50 (16%)	7/50 (14%)

B benign, *M* malignant

Table 18. Study 13–18-Month feeding study with glyphosate in mice (Feinchemie Schwebda 2001).

Study owner:	Feinchemie Schwebda (2001)					
Reliability/Justification	2 Study performed according to GLP and OECD guideline requirements, with no deviations, but possible viral infection may have confounded interpretation of results					
Substance:	Glyphosate (>95% pure)					
Species/Strain	Mouse/Swiss albino, groups of 50 ♂ and 50 ♀					
Administration route:	Diet					
Concentration:	0, 100, 1000, 10 000 ppm diet (♂ about 0, 14.5, 150, 1454 mg/kg bw/day; ♀ about 0, 15.0, 151, 1467 mg/kg bw/day)					
Duration:	18 months					
Findings:	1000 ppm diet: NOAEL (♂+♀) 10 000 ppm diet (♂+♀): increased mortality					
Select neoplasms:	Bronchiolar/alveolar adenoma, lymphoma					
	Historical controls		Dose (mg/kg bw/day)			
			0	14.5	150	1454
Males						
Mortality	§11/50–27/50		+22/50 (6)	20/50 (6)	22/50 (8)	27/50 (8)
Findings for dead and moribund sacrificed animals						
Lymphoma – M	#20/75	26.7% [0–44]	9/22 (41.0%)	*12/20 (60.0%)	*13/22 (59.0%)	13/27 (48.0%)
Findings in animals sacrificed at termination						
Lymphoma – M	26/175	14.9% [8–24]	1/28 (3.6%)	3/30 (10.0%)	3/28 (10.7%)	*6/23 (26.1%)
Total animals						
Lymphoma – M	46/250	18.4% [6–30]	10/50 (20.0%)	15/50 (30.0%)	16/50 (32.0%)	*19/50 (38.0%)
	Historical controls		Dose (mg/kg bw/day)			
			0	15.0	151	1467
Females						
Mortality	12/50–20/50		16/50 (7)	16/50 (7)	20/50 (2)	20/50 (3)
Findings for dead and moribund sacrificed animals						
Bronchiolar/alveolar adenoma – B	—	—	0/16	0/16	1/20 (5%)	2/20 (10%)
Lymphoma – M	49/77	63.6% [0–100]	9/16 (56.0%)	10/16 (63.0%)	13/20 (65.0%)	12/20 (60.0%)
Findings in animals sacrificed at termination						
Bronchiolar/alveolar adenoma – B			1/34 (3%)	0/0	1/1 (100%)	1/30 (3%)
Lymphoma – M	50/175	28.9% [2043]	9/34 (26.5%)	10/30 (29.4%)	6/30 (20.0%)	*13/28 (43.3%)
Total animals						
Bronchiolar/alveolar adenoma – B			1/50 (2%)	0/16	2/21 (10%)	3/50 (6%)
Lymphoma – M	99/250	39.6% [1458]	18/50 (36.0%)	20/50 (40.0%)	19/50 (38.0%)	*25/50 (50.0%)

B benign, *M* malignant.
§Nine studies, performed by the same laboratory in the timeframe encompassing the study summarized here.
+(Number of animals killed in extremis).
#Five studies, conducted in the same laboratory between 1996 and 1999.
*Statistically higher than concurrent controls ($p < 0.05$).

in the diet. Control mice received a plain diet. The calculated test substance intake was 14.5/15.0, 150/151, 1454/1467 mg/kg bw/day (males/females, Table 18), exceeding the limit dose, as reflected in elevated mortality in the high dose groups. This study was rated Klimisch 2 for reliability, based on speculation of a viral infection within the colony, discussed below.

Based on the slightly higher mortality and lower survival rates in the high dose groups, the NOAEL was considered 1000 ppm (151 mg/kg bw/day). There were no treatment-related effects on clinical signs, behavior, eyes, body weight, body weight gain, food consumption, and differential white blood cell counts in both sexes. Gross pathology, organ weight data, and histopathological examination demonstrated no treatment-related effects. An increase in the number of malignant lymphomas, the most common spontaneously occurring tumor category in the mouse, was statistically significant in the high-dose groups compared to controls (Table 18). The Germany Rapporteur Member State concluded that the malignant lymphoma increase in high-dose males was inconclusive but unrelated to treatment in the context of similar higher dosed studies (Germany Rapporteur Member State 2015b), and considered this endpoint irrelevant to carcinogenic risk in humans (Germany Rapporteur Member State 2015a). Whether or not a viral component (Taddesse-Heath et al. 2000) may have contributed to this endpoint, the finding was considered incidental background variation based on historical control data, and in agreement with the study director. As in Study 11, bronchiolar-alveolar adenoma was also considered a select neoplasm for evaluation in the broader data set (Tables 22 and 23), and as previously discussed, demonstrates a lack of dose-response across doses ranging from approximately 15 mg/kg bw/day to 5000 mg/kg bw/day. Summary tables of all histopathological neoplastic findings are available (see data Supplementary Study 13 to be found online at http://informahealthcare.com/doi/abs/10.3109/10408444.2014.1003423).

Technical grade glyphosate was reported as not carcinogenic in Swiss albino mice, following continuous dietary exposure of up to 1460 mg/kg bw/day (average for both sexes) for 18 months. The NOAEL for general chronic toxicity was 151 mg/kg bw/day for both sexes combined.

Study 14 (Nufarm 2009a)

The most recent mouse carcinogenicity assay was conducted between October 2005 and November 2007 (Nufarm 2009a).

 Case 3:16-md-02741-VC Document 654-2 Filed 10/28/17 Page 40 of 138

Table 19. Study 14–18-Month feeding study with glyphosate in mice (Nufarm 2009a).

Study owner:	Nufarm (2009b)			
Reliability/Justification:	1 Study performed according to GLP and OECD guideline requirements, with no deviations			
Substance:	Glyphosate (94.6–97.6% pure)			
Species/Strain:	mouse/CD-1, groups of 51 ♂ and 51 ♀			
Administration route:	Diet			
Concentration:	0, 500, 1500, and 5000 ppm diet (♂ about 0, 0, 71.4, 234, 810 mg/kg bw/day; ♀ about 0, 97.9, 300, 1081 mg/kg bw/day)			
Duration:	18 months			
Findings:	≥ 5000 ppm diet: NOAEL (♂/♀) No treatment-related effects			
Select neoplasms:	Bronchiolar-alveolar adenoma, Bronchiolar-alveolar adenocarcinoma, hepatocellular adenoma (males), hepatocellular carcinoma (males), lymphoma, pituitary adenoma (females)			
		Dose (mg/kg bw/day)		
Males	0	157	814	4841
Bronchiolar-alveolar adenoma – B	9/51 (18%)	7/51 (14%)	9/51 (18%)	4/51 (8%)
Bronchiolar-alveolar adenocarcinoma – M	5/51 (10%)	5/51 (10%)	7/51 (14%)	11/51 (22%)
Hepatocellular adenoma – B	1/51 (2%)	1/51 (2%)	4/51 (8%)	2/51 (4%)
Hepatocellular carcinoma – M	6/51 (12%)	11/51 (22%)	7/51 (14%)	4/51 (8%)
Lymphoma – M	0/51	1/50 (2%)	2/51 (4%)	5/51 (10%)
		Dose (mg/kg bw/day)		
Females	0	190	955	5873
Bronchiolar-alveolar adenoma – B	2/51 (4%)	4/51 (8%)	2/51 (4%)	2/51 (4%)
Bronchiolar-alveolar adenocarcinoma – M	5/51 (10%)	2/51 (4%)	2/51 (4%)	3/51 (6%)
Lymphoma – M	11/51 (22%)	8/51 (16)	10/51 (20%)	11/51 (22%)
Pituitary adenoma – B	0/51	1/50 (2%)	0/51	2/51 (4%)

B benign, *M* malignant

Groups of 51 CD-1 mice per sex received daily dietary doses of 0, 500, 1500, and 5000 ppm technical grade glyphosate (equivalent to an average intake of 85, 267 and 946 mg/kg bw/day, Table 19). The MTD was apparently not reached in the high-dose group, which is more indicative of low general toxicity of the test substance rather than a flaw in the study design. The NOAEL for chronic toxicity was 810 mg/kg bw/day for male mice and 1081 mg/kg bw/day for female mice, the highest dosage tested. Despite not quite achieving a limit dose in males, this study was arguably rated Klimisch 1 for reliability.

Several increases in common spontaneous mouse neoplasms in male mice were noted. Non-dose-response increases were noted for hepatocellular adenoma and carcinoma in males, and dose-responses were noted for bronchiolar-alveolar adenocarcinoma and malignant lymphoma in males, but not females. Pituitary adenoma incidences were low, and considered incidental in low and high-dose females, although they were slightly higher than controls (Table 19). These neoplasms were all evaluated in context of the broader data set (Tables 22 and 23). The summary of neoplastic findings is available (see data Supplementary Study 14 to be found online at http://informahealthcare.com/doi/abs/10.3109/10408444.2014.1003423).

Glyphosate was considered not carcinogenic in the CD-1 mice, following continuous average dietary exposure for males and females, to quantities up to 945.6 mg/kg bw/day for 18 months, which is consistent with the recent evaluation in Europe under the Annex I Renewal of glyphosate (Germany Rapporteur Member State 2015b).

Discussion

An extraordinarily large volume of animal data has been compiled to evaluate the carcinogenic potential of glyphosate. The expected normal biological variability for spontaneous tumor formation is reflected across this extensive data set (Tables 20–23). However, no specific neoplasm stands out as a consequence of glyphosate exposures. While some individual studies may note an increase in a specific neoplasm at the high dose, the pooled data fail to identify any consistent pattern of neoplasm formation, demonstrating that the effect is not reproducible and not treatment-related. The lack of a dose-response across the several orders of magnitude suggests that no individual tumor of single etiology is attributable to glyphosate administration.

Glyphosate has undergone repeated and extensive review by the United States Environmental Protection Agency (US EPA 1993), the European Union (EC 2002, Germany Rapporteur Member State 2015b) and the World Health Organization/Food and Agriculture Organization of the United Nations (WHO/FAO 2004b, WHO/FAO 2004a). With regard to potential carcinogenic effects of glyphosate, the unanimous outcome of these reviews has been that the data provide sufficient evidence to conclude that glyphosate should not be considered a carcinogen. Genotoxicity studies with glyphosate, conducted under conditions stipulated by internationally accepted testing guidelines and GLP, as reviewed in 2000 (Williams et al. 2000) and recently updated (Kier and Kirkland 2013), indicate that glyphosate clearly does not exhibit the properties of a DNA-reactive genotoxic carcinogen. This lack of mutagenicity rules out an important concern for carcinogenicity.

Mink et al. published a review of the available epidemiological studies that investigated possible associations between glyphosate and cancer diagnosed in humans (Mink et al. 2012). No evidence was found for a statistically significant positive association between cancer and exposure to glyphosate. While one Agricultural Health Study (AHS) publication mentions a "suggested association" between glyphosate use and multiple myeloma (De Roos et al. 2005), a later summary of AHS

Case 3:16-md-02741-VC Document 654-2 Filed 10/28/17 Page 41 of 138

Table 20. Summary of select neoplasms in male rats (Studies 1–8).

Select neoplasm	Controls – 0 [% range for studies]	Tumor Incidence/number of animals examined, by dose (mg/kg bw/day)										
		[a]3	[d]7.4	[a]10	[c]10	[a]31	[d]73.9	[h]86	[b]89	[c]100	[f]104	[g]121
Pancreas islet cell adenoma	20/397 [0–14]	5/49	0/30	2/50	1/24	2/50	0/32	1/51	8/57	2/17	1/75	2/64
Pituitary adenoma	153/398 [6–57]	19/49	4/30	20/48	12/24	18/47	3/31	11/51	32/58	8/19	41/75	17/63
Pituitary carcinoma	4/98 [2–6]	2/49	NF	3/48	1/24	1/47	NF	NF	NF	0/19	NF	NF
Testes interstitial cell (Leydig)	14/447 [0–8]	3/50	0/37	1/50	1/25	6/50	2/32	3/51	0/60	0/19	2/75	2/63
Thyroid C cell adenoma	35/391 [4–18]	1/49	0/26	0/49	1/21	2/49	1/29	#1/51	5/58	1/17	10/74	#1/63
Hepatocellular adenoma	30/351 [0–48]	NF	22/50	NF	1/50	NF	10/48	2/51	2/60	1/49	0/75	2/64
Hepatocellular carcinoma	22/384 [0–42]	0/50	28/50	1/50	1/50	2/50	18/48	0/51	2/60	1/49	1/75	NF
Benign keratoacanthoma (skin)	8/250 [2–5]	NF	NF	NF	NF	NF	NF	3/51	3/60	NF	3/75	0/64

Select neoplasm	Tumor Incidence/number of animals examined, by dose (mg/kg bw/day)												
	[e]150	[h]285	[c]300	[f]354	[g]361	[b]362	[d]740.6	[e]780	[b]940	[c]1000	[h]1077	[f]1127	[g]1214	[e]1290
Pancreas islet cell adenoma	NF	2/51	2/21	1/80	0/64	5/60	1/49	NF	7/59	1/49	1/51	1/78	1/64	NF
Pituitary adenoma	NF	10/51	7/21	33/80	18/64	34/58	5/49	NF	32/59	17/50	20/51	42/78	19/63	NF
Pituitary carcinoma	NF	NF	1/21	NF	NF	NF	NF	NF	NF	0/50	NF	NF	NF	NF
Testes interstitial cell (Leydig)	1/49	1/51	0/21	0/80	2/63	3/60	3/50	2/49	2/60	2/50	1/51	2/78	2/64	0/47
Thyroid C cell adenoma	NF	#0/51	2/21	5/79	#1/63	8/58	1/50	NF	7/60	8/49	#3/51	6/78	#0/64	NF
Hepatocellular adenoma	NF	0/51	2/50	2/80	0/64	3/60	21/50	NF	8/60	2/50	1/51	1/78	5/64	NF
Hepatocellular carcinoma	1/49	0/51	0/50	2/80	NF	1/60	24/50	0/49	2/60	0/50	0/51	1/78	NF	0/47
Benign keratoacanthoma (skin)	NF	0/51	NF	0/80	1/64	4/60	NF	NF	5/59	NF	6/51	7/78	1/63	NF

[a]Study 1 (Monsanto) (CD) SD rats, rated unreliable for carcinogenicity evaluation.
[b]Study 2 (Monsanto) (CD) SD rats, including interim sacrifice groups.
[c]Study 3 (Cheminova) SD rats.
[d]Study 4 (Feinchemic Schwebda) Wistar rats.
[e]Study 5 (Excel) SD rats. rated unreliable for carcinogenicity evaluation.
[f]Study 6 (Arysta Life Sciences) Crj:CD SD rats, including interim sacrifice groups.
[g] Study 7 (Syngenta) Alpk:AP$_f$SD Wistar rats, including interim sacrifice groups.
[h] Study 8 (Nufarm) Wistar Han Crl:WI rats.
[#]Recorded as parafollicular adenoma.
NF not found/not reported

Table 21. Summary of select neoplasms in female rats (Studies 1–8).

Select neoplasm	Controls – 0 [% range for studies]	Tumor Incidence/number of animals examined, by dose (mg/kg bw/day)										
		[a]3	[d]7.4	[c]10	[a]11	[a]34	[d]73.9	[c]100	[h]105	[b]113	[f]115	[g]145
Pancreas islet cell adenoma	11/397 [0–9]	1/50	0/23	2/27	1/50	0/49	0/16	2/29	0/51	1/60	2/79	0/63
Pituitary adenoma	246/397 [14–78]	29/48	13/33	19/28	31/50	26/49	7/23	19/29	23/51	48/60	54/79	44/63
Pituitary carcinoma	16/155 [2–17]	7/48	NF	5/28	5/50	12/49	NF	5/28	NF	0/60	NF	NF
Thyroid C cell adenoma	25/302 [3% – 16%]	3/49	0/24	1/27	6/50	3/47	1/17	1/29	# 1/51	2/60	7/78	# 0/63
Hepatocellular adenoma	22/302 [0–36]	NF	18/48	1/50	NF	NF	19/49	3/50	0/51	2/60	1/79	0/64
Hepatocellular carcinoma	14/210 [0–20]	0/50	15/48	0/50	0/50	2/50	14/49	0/50	0/51	0/60	NF	NF
Mammary gland fibroadenoma	113/384 [6–58]	16/46	NF	12/28	20/48	16/44	NF	17/29	9/51	$24/54	30/79	4/63
Mammary gland adenocarcinoma	40/334 [2–22]	6/46	0/30	NF	5/48	8/44	0/33	NF	3/51	~10/54	8/79	0/63

Select neoplasm	Tumor Incidence/number of animals examined, by dose (mg/kg bw/day)												
	[e]210	[c]300	[h]349	[f]393	[g]437	[b]457	[d]740.6	[c]1000	[e]1060	[b]1183	[f]1247	[h]1382	[g]1498	[e]1740
Pancreas islet cell adenoma	NF	2/29	0/51	1/78	1/64	4/60	1/49	1/49	NF	0/59	1/78	0/51	0/64	NF
Pituitary adenoma	NF	25/30	16/51	47/77	46/63	46/60	6/50	34/49	NF	34/59	52/78	32/51	49/64	NF
Pituitary carcinoma	NF	2/30	NF	NF	NF	0/60	NF	7/49	NF	1/59	NF	NF	NF	NF
Thyroid C cell adenoma	NF	2/29	#1/50	8/76	#0/64	6/60	1/47	7/49	NF	6/60	4/78	# 0/51	# 2/64	NF
Hepatocellular adenoma	NF	1/50	1/51	0/78	1/64	6/60	13/50	2/50	NF	1/60	0/78	1/51	0/64	NF
Hepatocellular carcinoma	NF	0/50	1/51	NF	NF	1/60	9/50	0/50	NF	2/60	NF	0/51	NF	NF
Mammary gland fibroadenoma	1/22	19/30	7/51	27/77	6/64	$27/59	NF	29/50	5/22	$28/57	30/78	5/51	5/64	5/50
Mammary gland adenocarcinoma	0/22	NF	1/51	11/77	0/64	~14/59	0/48	NF	0/22	~9/57	8/78	6/51	2/64	0/50

[a]Study 1 (Monsanto) (CD) SD rats, rated unreliable for carcinogenicity evaluation.
[b]Study 2 (Monsanto) (CD) SD rats, including interim sacrifice groups.
[c]Study 3 (Cheminova) SD rats.
[d]Study 4 (Feinchemic Schwebda) Wistar rats.
[e]Study 5 (Excel) SD rats, rated unreliable for carcinogenicity evaluation.
[f]Study 6 (Arysta Life Sciences) Crj:CD SD rats, including interim sacrifice groups.
[g]Study 7 (Syngenta) Alpk:AP$_f$SD Wistar rats, including interim sacrifice groups.
[h]Study 8 (Nufarm) Wistar Han Crl:WI rats.
[$]Recorded as adenoma/adenofibroma/fibroma.
[~]Recorded as carcinoma/adenocarcinoma.
NF not found/not reported.

 Case 3:16-md-02741-VC Document 654-2 Filed 10/28/17 Page 42 of 138

Table 22. Summary of select neoplasms in male mice (Studies 10–14).

	Tumor Incidence/number of animals examined, by dose (mg/kg bw/day)							
Select neoplasm	Controls – 0 [% range for studies]	[d]14.5	[e]85	[b]100	[d]150	[a]157	[c]165	[e]267
Bronchiolar-alveolar adenoma	31/249 [10–18]	2/22	[§]7/51	15/50	0/22	9/50	[§]14/50	[§]9/51
Bronchiolar-alveolar adenocarcinoma	10/149 [2–10]	NF	[§]5/51	NF	NF	3/50	[§]1/50	[§]7/51
Bronchiolar-alveolar carcinoma	10/100 [0–20]	0/22	NF	7/50	0/22	NF	NF	NF
Hepatocellular adenoma	27/250 [0–28]	5/25	1/51	12/50	3/28	0/50	15/50	4/51
Hepatocellular carcinoma	15/250 [0–16]	0/25	11/51	5/50	0/28	0/50	1/50	7/51
Malignant lymphoma	16/205 [0–100]	15/50	1/51	2/4	16/50	[#]5/50	2/50	2/51
Myeloid leukemia	3/101 [0–6]	1/50	1/51	NF	1/50	NF	NF	0/51
	Tumor Incidence/number of animals examined, by dose (mg/kg bw/day)							
Select neoplasm	[b]300	[a]814	[c]838	[e]946	[b]1000	[d]1454	[c]4348	[a]4841
Bronchiolar-alveolar adenoma	11/50	9/50	[§]13/50	[§]4/51	13/50	1/50	[§]11/50	9/50
Bronchiolar-alveolar adenocarcinoma	NF	2/50	[§]6/50	[§]11/51	NF	NF	[§]4/50	1/50
Bronchiolar-alveolar carcinoma	8/50	NF	NF	NF	9/50	1/50	NF	NF
Hepatocellular adenoma	11/50	1/50	15/50	2/51	9/50	3/50	7/50	0/50
Hepatocellular carcinoma	6/50	0/50	3/50	4/51	7/50	2/50	1/50	2/50
Malignant lymphoma	1/1	[#]4/50	0/50	5/51	6/8	19/50	6/50	[#]2/50
Myeloid leukemia	NF	NF	NF	0/51	NF	1/50	NF	NF

[a]Study 10 (Monsanto) CD-1 mice.
[b]Study 11 (Cheminova) CD-1 mice.
[c]Study 12 (Arysta Life Science) CD-1 mice.
[d]Study 13 (Feinchemie Schwebda) Swiss albino mice.
[e]Study 14 (Nufarm) CD-1 mice.
[§]Recorded as lung rather than bronchiolar-alveolar.
[#]Recorded as sum of malignant lymphoblastic lymphosarcoma with leukemia, lymphoblastic lymphosarcoma without leukemia and composite lymphosarcoma.
[$]Recorded as lymphoblastic lymphosarcoma with leukemia.
NF not found/not reported.

results note that there were no associations between glyphosate use and a number of cancers, including lymphohematopoietic cancers, leukemia, NHL, and multiple myeloma (Weichenthal et al. 2010). A subsequent reanalysis of AHS data obtained under the Freedom of Information Act notes no suggestion of an association between glyphosate use and multiple myeloma, with a relative risk of 1.1 and 95% and a confidence interval of 0.5–2.9 (Sorahan 2012). A recent review paper (Alavanja et al. 2013) cites another epidemiology study claiming an association between glyphosate use and NHL (Eriksson et al. 2008), but this research is strongly criticized in the recent Reevaluation Assessment Report for glyphosate Annex I Renewal in Europe (Germany Rapporteur Member State 2015b), highlighting potential referral bias, selection bias, uncontrolled confounding, limited data usage contrary to claims of including all new cases (living cases only, rather than living

Table 23. Summary of select neoplasms in female mice (Studies 10–14).

	Tumor incidence/number of animals examined, by dose (mg/kg bw/day)							
Select neoplasm	Controls – 0 [% range for studies]	[d]15.0	[e]85	[b]100	[d]151	[c]153	[a]190	[e]267
Bronchiolar-alveolar adenoma	28/250 [2–20]	0/16	[§]4/51	3/49	2/21	[§]5/50	9/50	[§]2/51
Bronchiolar-alveolar adenocarcinoma	2/99 [2]	NF	[§]2/51	NF	NF	[§]2/50	3/50	[§]2/51
Bronchiolar-alveolar carcinoma	9/151 [2–10]	0/16	NF	2/49	0/20	NF	NF	NF
Malignant lymphoma	54/215 [10–100]	20/50	8/51	12/15	19/50	4/50	[#]6/50	10/51
Myeloid leukemia	2/156 [0–4]	1/50	0/51	NF	2/50	0/50	NF	1/51
Pituitary adenoma	1/232 [0–2]	0/16	1/51	0/32	0/17	1/50	0/21	0/51
	Tumor incidence/number of animals examined, by dose (mg/kg bw/day)							
Select neoplasm	[b]300	[c]787	[e]946	[a]955	[b]1000	[d]1467	[c]4116	[a]5874
Bronchiolar-alveolar adenoma	3/50	[§]12/50	[§]2/51	10/49	6/50	3/50	[§]5/50	1/50
Bronchiolar-alveolar adenocarcinoma	NF	[§]3/50	[§]3/51	4/49	NF	NF	[§]1/50	4/50
Bronchiolar-alveolar carcinoma	1/50	NF	NF	NF	5/50	0/50	NF	NF
Malignant lymphoma	9/12	8/50	11/51	[#]6/50	13/14	25/50	7/50	[#]10/50
Myeloid leukemia	NF	0/50	0/51	NF	NF	1/50	1/50	NF
Pituitary adenoma	0/23	0/50	2/51	0/44	~3/50	1/48	0/50	0/37

[a]Study 10 (Monsanto) CD-1 mice.
[b]Study 11 (Cheminova) CD-1 mice.
[c]Study 12 (Arysta Life Science) CD-1 mice.
[d]Study 13 (Feinchemie Schwebda) Swiss albino mice.
[e]Study 14 (Nufarm) CD-1 mice.
[§]Recorded as lung rather than bronchiolar-alveolar.
[#]Recorded as sum of lymphoblastic lymphosarcoma with leukemia, lymphoblastic lymphosarcoma without leukemia and composite lymphosarcoma.
~2 animals in anterior lobe, 1 animal in intermediate lobe.
NF not found/not reported.

Case 3:16-md-02741-VC Document 654-2 Filed 10/28/17 Page 43 of 138

plus dead), and questionable definition/interpretation of dose-response. It is important to note that the Eriksson et al. study did detect statistically significant positive associations for small lymphocytic lymphoma/chronic lymphocytic leukemia and "unspecified NHL", while the following lymphomas were not statistically significantly associated with glyphosate use: B-cell lymphomas, grade I-III follicular lymphoma, diffuse large B-cell lymphoma, other specified B-cell lymphomas, unspecified B cell lymphomas, and T-cell lymphomas (Eriksson et al. 2008). As previously discussed, statistically significant associations need to be evaluated further for study bias, confounders and sampling error, before expending resources and energy on further evaluation of potential causality.

Epidemiological investigations face the difficulty of reliably determining the magnitude of exposure to the chemical in question, while ruling out confounders like co-exposure to other chemicals, and environmental and lifestyle factors. In contrast, carcinogenicity studies in experimental animals, when conducted according to appropriate testing guidelines, are designed in a fashion that allows a direct association between observed effects and substance exposure, yet the relevance of observed findings to humans is an important consideration. This manuscript collectively presents the scientific community with carcinogenicity results from a remarkably large body of data from fourteen long-term carcinogenicity studies on glyphosate.

Glyphosate is of very low acute toxicity with an oral LD_{50} in the rat in excess of 5000 mg/kg of body weight.. The sub-chronic NOAEL is 400 mg/kg bw/day, and is based on effects that do not impair long-term survival (WHO/FAO 2004b, WHO/FAO 2004a). This allows administration of very high glyphosate doses to rodents for a prolonged time. Dietary levels of up to 30 000 and 40 000 milligrams of glyphosate per kilogram of diet have been administered to rats and mice, respectively, in chronic feeding studies covering their expected lifespan without apparent effects on longevity.

One of the most critical aspects of designing a carcinogenicity study is the choice of dose levels, especially the top dose, at either the limit dose or MTD. The relevant OECD TGs 451 and 453 for carcinogenicity studies propose a body weight depression of approximately 10% as evidence for systemic toxicity. This is equivalent to the concept of the MTD, which is discussed in a supporting OECD guidance document (OECD 2012b). For chemicals which are well tolerated by the experimental animal, where no dose-limiting toxicity is observed, the respective OECD guidance suggests 1000 mg/kg bw/day as the highest dose level (OECD 2012a). Many of the carcinogenicity studies performed in rats and mice with glyphosate have been conducted with the high dose group receiving levels of glyphosate at, or in excess of the limit dose because of its very low toxicity following repeat exposure. Following this extensive testing, even at very high exposure levels, there was no evidence of a carcinogenic effect related to glyphosate treatment. The select neoplasms highlighted in Tables 20–23 show normal biological background levels of spontaneous neoplasms, with lack of dose-response across the data sets. The combined studies clearly indicate that glyphosate's carcinogenic potential is extremely low or non-existent in animal models up to very high doses.

By way of comparison, the worst-case calculated human dietary exposure to glyphosate, the Theoretical Maximum Daily Intake (TMDI) is 0.14 mg/kg bw/day (EFSA 2012). Systemic exposure of operators, as assessed for the EU reapproval of glyphosate, is predicted to be between 0.0034 (German BBA model, tractor-mounted ground-boom sprayer) and 0.226 mg/kg bw/day (UK POEM, hand-held-spraying to low targets, data not shown). The model estimates are supported by human biomonitoring data in farmers showing systemic exposures of 0.004 and 0.0001 mg/kg/day for worst-case and mean acute doses, respectively (Acquavella et al. 2004). The high doses in chronic rodent studies at which no evidence of carcinogenicity is demonstrated are at least hundreds of thousands fold greater than peak human systemic exposure levels. Clearly, there is no scientific basis for concern of carcinogenic risk to humans resulting from glyphosate exposure.

With over 40 years of scientific research on glyphosate, no compelling evidence exists for a mechanism for glyphosate to cause cancer. Mammalian metabolism does not activate glyphosate to a toxic metabolite (Anadon et al. 2009, WHO/FAO 2004a). The lack of glyphosate DNA reactivity supports the

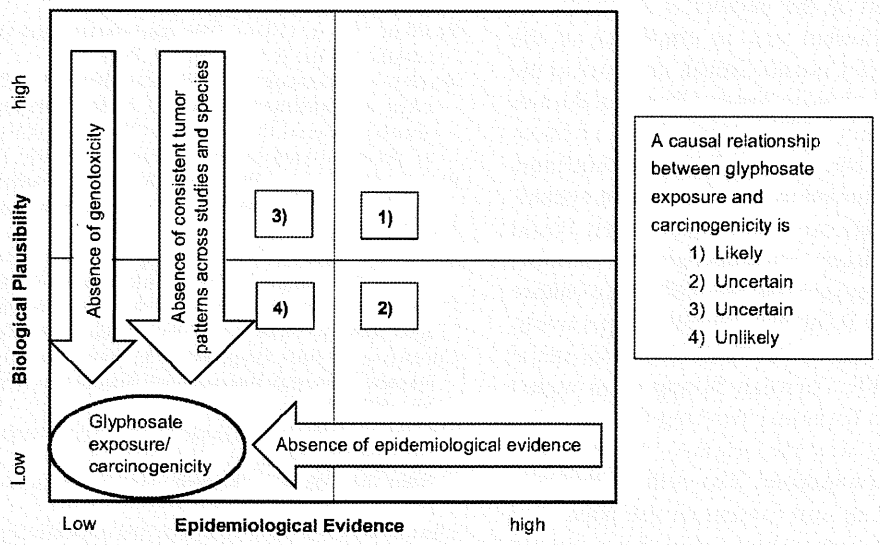

Figure 2. Likelihood of glyphosate carcinogenicity based on experimental and epidemiological data; a causal inference grid as proposed by Adami et al. (2011) to utilize both toxicological and epidemiological data.

lack of potential for an initiation event for carcinogenesis (Kier and Kirkland 2013). Clearly, there is a lack of potential for glyphosate to induce hormonal oncogenesis, based on both the tumor incidence data presented and the unequivocal evidence that glyphosate is not an endocrine disruptor (Bailey et al. 2013, Levine et al. 2012, Saltmiras and Tobia 2012, Webb et al. 2013, Williams et al. 2012).

The absence of test substance-related neoplastic findings in a total of 14 rodent cancer bioassays with glyphosate is in stark contrast to the recent dramatic media reports, internet postings, and YouTube videos of rat tumors, hypothesized to be caused by treatment with maize containing glyphosate residue or drinking water spiked with a glyphosate formulation (Seralini et al. 2014). Such reports, under the scrutiny of the global scientific community, demand greater data transparency and accountability within the peer review process.

The absence of a glyphosate-related mechanism for carcinogenesis, the huge volume of genotoxicity data studies indicating no likely mutagenic or DNA-reactive potential (Kier and Kirkland 2013), combined with the lack of epidemiological evidence for glyphosate-induced cancer (Mink et al. 2012), and the lack of carcinogenicity in multiple rodent carcinogenicity assays, are depicted in a causal inference grid in Figure 2, as put forth by Adami et al. (Adami et al. 2011). The overwhelming weight of the available evidence, demonstrating a lack of both biological plausibility and epidemiological effects, draws a compelling conclusion that glyphosate's carcinogenic potential is extremely low or non-existent.

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Declaration of interest

The employment affiliation of the authors is as shown on the cover page. Volker Mostert was an employee of the consulting group, Dr. Knoell Consult GmbH, involved in the preparation of the recent glyphosate Annex I Renewal dossier for the Glyphosate Task Force (GTF; a consortium of European glyphosate registrants http://www.glyphosatetaskforce.org/). Helmut Greim was funded as an independent consultant for his expert contributions to this manuscript. David Saltmiras and Christian Strupp are employed by member companies of the GTF, Monsanto and ADAMA Agriculture B.V. (formerly Feinchemie Schwebda GmbH) respectively. David Saltmiras is also Chair of the Toxicology Technical Working Group of the GTF. Christian Strupp is an expert member of the Toxicology Technical Working Group of the GTF. Monsanto Company was the original producer and marketer of glyphosate formulations. The authors had sole responsibility for the writing and content of the paper and the interpretations and opinions expressed in the paper are those of the authors and may not necessarily be those of the member companies of the Glyphosate Task Force.

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Case 3:16-md-02741-VC Document 654-2 Filed 10/28/17 Page 46 of 138

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Supplementary material available online

Data Supplementary Study 1–14.

Nabhan
EXHIBIT NO. 6
8/22/17
P. CAMPBELL

GLYPHOSATE

1. Exposure Data

1.1 Identification of the agent

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 1071-83-6 (acid); also relevant:
38641-94-0 (glyphosate-isopropylamine salt)
40465-66-5 (monoammonium salt)
69254-40-6 (diammonium salt)
34494-03-6 (glyphosate-sodium)
81591-81-3 (glyphosate-trimesium)

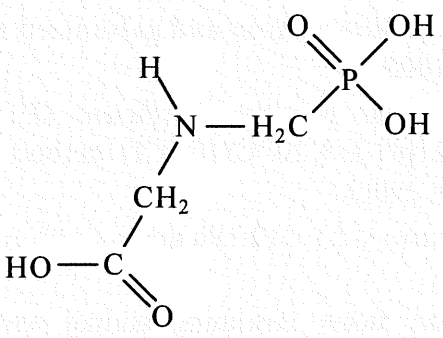
Chem. Abstr. Serv. Name: *N*-(phosphonomethyl)glycine

Preferred IUPAC Name: *N*-(phosphonomethyl)glycine

Synonyms: Gliphosate; glyphosate; glyphosate hydrochloride; glyphosate [calcium, copper (2+), dilithium, disodium, magnesium, monoammonium, monopotassium, monosodium, sodium, or zinc] salt

Trade names: Glyphosate products have been sold worldwide under numerous trade names, including: Abundit Extra; Credit; Xtreme; Glifonox; Glyphogan; Ground-Up; Rodeo; Roundup; Touchdown; Tragli; Wipe Out; Yerbimat (Farm Chemicals International, 2015).

1.1.2 Structural and molecular formulae and relative molecular mass



Molecular formula: $C_3H_8NO_5P$
Relative molecular mass: 169.07

Additional information on chemical structure is also available in the PubChem Compound database (NCBI, 2015).

1.1.3 Chemical and physical properties of the pure substance

Description: Glyphosate acid is a colourless, odourless, crystalline solid. It is formulated as a salt consisting of the deprotonated acid of glyphosate and a cation (isopropylamine, ammonium, or sodium), with more than one salt in some formulations.

Solubility: The acid is of medium solubility at 11.6 g/L in water (at 25 °C) and insoluble in common organic solvents such as acetone, ethanol, and xylene; the alkali-metal and

amine salts are readily soluble in water (Tomlin, 2000).

Volatility: Vapour pressure, 1.31×10^{-2} mPa at 25 °C (negligible) (Tomlin, 2000).

Stability: Glyphosate is stable to hydrolysis in the range of pH 3 to pH 9, and relatively stable to photodegradation (Tomlin, 2000). Glyphosate is not readily hydrolysed or oxidized in the field (Rueppel *et al.* 1977). It decomposes on heating, producing toxic fumes that include nitrogen oxides and phosphorus oxides (IPCS, 2005).

Reactivity: Attacks iron and galvanized steel (IPCS, 2005).

Octanol/water partition coefficient (P): log P, < –3.2 (pH 2–5, 20 °C) (OECD method 107) (Tomlin, 2000).

Henry's law: $< 2.1 \times 10^{-7}$ Pa m^3 mol^{-1} (Tomlin, 2000).

Conversion factor: Assuming normal temperature (25 °C) and pressure (101 kPa), $mg/m^3 = 6.92 \times$ ppm.

1.1.4 Technical products and impurities

Glyphosate is formulated as an isopropylamine, ammonium, or sodium salt in water-soluble concentrates and water-soluble granules. The relevant impurities in glyphosate technical concentrates are formaldehyde (maximum, 1.3 g/kg), *N*-nitrosoglyphosate (maximum, 1 mg/kg), and *N*-nitroso-*N*-phosphonomethylglycine (FAO, 2000). Surfactants and sulfuric and phosphoric acids may be added to formulations of glyphosate, with type and concentration differing by formulation (IPCS, 1994).

1.2 Production and use

1.2.1 Production

(a) Manufacturing processes

Glyphosate was first synthesized in 1950 as a potential pharmaceutical compound, but its herbicidal activity was not discovered until it was re-synthesized and tested in 1970 (Székács & Darvas, 2012). The isopropylamine, sodium, and ammonium salts were introduced in 1974, and the trimesium (trimethylsulfonium) salt was introduced in Spain in 1989. The original patent protection expired outside the USA in 1991, and within the USA in 2000. Thereafter, production expanded to other major agrochemical manufacturers in the USA, Europe, Australia, and elsewhere (including large-scale production in China), but the leading preparation producer remained in the USA (Székács & Darvas, 2012).

There are two dominant families of commercial production of glyphosate, the "alkyl ester" pathways, predominant in China, and the "iminodiacetic acid" pathways, with iminodiacetic acid produced from iminodiacetonitrile (produced from hydrogen cyanide), diethanol amine, or chloroacetic acid (Dill *et al.*, 2010; Tian *et al.*, 2012).

To increase the solubility of technical-grade glyphosate acid in water, it is formulated as its isopropylamine, monoammonium, potassium, sodium, or trimesium salts. Most common is the isopropylamine salt, which is formulated as a liquid concentrate (active ingredient, 5.0–62%), ready-to-use liquid (active ingredient, 0.5–20%), pressurized liquid (active ingredient, 0.75–0.96%), solid (active ingredient, 76–94%), or pellet/tablet (active ingredient, 60–83%) (EPA, 1993a).

There are reportedly more than 750 products containing glyphosate for sale in the USA alone (NPIC, 2010). Formulated products contain various non-ionic surfactants, most notably polyethyloxylated tallowamine (POEA), to

facilitate uptake by plants (Székács & Darvas, 2012). Formulations might contain other active ingredients, such as simasine, 2,4-dichlorophenoxyacetic acid (2,4-D), or 4-chloro-2-methylphenoxyacetic acid (IPCS, 1996), with herbicide resistance driving demand for new herbicide formulations containing multiple active ingredients (Freedonia, 2012).

(b) Production volume

Glyphosate is reported to be manufactured by at least 91 producers in 20 countries, including 53 in China, 9 in India, 5 in the USA, and others in Australia, Canada, Cyprus, Egypt, Germany, Guatemala, Hungary, Israel, Malaysia, Mexico, Singapore, Spain, Taiwan (China), Thailand, Turkey, the United Kingdom, and Venezuela (Farm Chemicals International, 2015). Glyphosate was registered in over 130 countries as of 2010 and is probably the most heavily used herbicide in the world, with an annual global production volume estimated at approximately 600 000 tonnes in 2008, rising to about 650 000 tonnes in 2011, and to 720 000 tonnes in 2012 (Dill *et al.*, 2010; CCM International, 2011; Hilton, 2012; Transparency Market Research, 2014).

Production and use of glyphosate have risen dramatically due to the expiry of patent protection (see above), with increased promotion of non-till agriculture, and with the introduction in 1996 of genetically modified glyphosate-tolerant crop varieties (Székács & Darvas, 2012). In the USA alone, more than 80 000 tonnes of glyphosate were used in 2007 (rising from less than 4000 tonnes in 1987) (EPA, 1997, 2011). This rapid growth rate was also observed in Asia, which accounted for 30% of world demand for glyphosate in 2012 (Transparency Market Research, 2014). In India, production increased from 308 tonnes in 2003–2004, to 2100 tonnes in 2007–2008 (Ministry of Chemicals & Fertilizers, 2008). China currently produces more than 40% of the global supply of glyphosate, exports almost 35% of the global supply (Hilton, 2012), and reportedly has sufficient production capacity to satisfy total global demand (Yin, 2011).

1.2.2 Uses

Glyphosate is a broad-spectrum, post-emergent, non-selective, systemic herbicide, which effectively kills or suppresses all plant types, including grasses, perennials, vines, shrubs, and trees. When applied at lower rates, glyphosate is a plant-growth regulator and desiccant. It has agricultural and non-agricultural uses throughout the world.

(a) Agriculture

Glyphosate is effective against more than 100 annual broadleaf weed and grass species, and more than 60 perennial weed species (Dill *et al.*, 2010). Application rates are about 1.5–2 kg/ha for pre-harvest, post-planting, and pre-emergence use; about 4.3 kg/ha as a directed spray in vines, orchards, pastures, forestry, and industrial weed control; and about 2 kg/ha as an aquatic herbicide (Tomlin, 2000). Common application methods include broadcast, aerial, spot, and directed spray applications (EPA, 1993a).

Due to its broad-spectrum activity, the use of glyphosate in agriculture was formerly limited to post-harvest treatments and weed control between established rows of tree, nut, and vine crops. Widespread adoption of no-till and conservation-till practices (which require chemical weed control while reducing soil erosion and labour and fuel costs) and the introduction of transgenic crop varieties engineered to be resistant to glyphosate have transformed glyphosate to a post-emergent, selective herbicide for use on annual crops (Duke & Powles, 2009; Dill *et al.* 2010). Glyphosate-resistant transgenic varieties have been widely adopted for the production of corn, cotton, canola, and soybean (Duke & Powles, 2009). Production of such crops accounted for 45% of worldwide demand for glyphosate in 2012 (Transparency Market Research, 2014). However, in Europe,

where the planting of genetically modified crops has been largely restricted, post-harvest treatment is still the most common application of glyphosate (Glyphosate Task Force, 2014). Intense and continuous use of glyphosate has led to the emergence of resistant weeds that may reduce its effectiveness (Duke & Powles, 2009).

(b) Residential use

Glyphosate is widely used for household weed control throughout the world. In the USA, glyphosate was consistently ranked as the second most commonly used pesticide (after 2,4-D) in the home and garden market sector between 2001 and 2007, with an annual use of 2000–4000 tonnes (EPA, 2011).

(c) Other uses

Glyphosate was initially used to control perennial weeds on ditch banks and roadsides and under power lines (Dill *et al.*, 2010). It is also used to control invasive species in aquatic or wetland systems (Tu *et al.*, 2001). Approximately 1–2% of total glyphosate use in the USA is in forest management (Mance, 2012).

Glyphosate has been used in a large-scale aerial herbicide-spraying programme begun in 2000 to reduce the production of cocaine in Colombia (Lubick, 2009), and of marijuana in Mexico and South America (Székács & Darvas, 2012).

(d) Regulation

Glyphosate has been registered for use in at least 130 countries (Dill *et al.*, 2010). In the USA, all uses are eligible for registration on the basis of a finding that glyphosate "does not pose unreasonable risks or adverse effects to humans or the environment" (EPA, 1993a). A review conducted in 2001 in connection with the registration process in the European Union reached similar conclusions regarding animal and human safety, although the protection of groundwater during non-crop use was identified as requiring particular attention in the short term (European Commission, 2002).

Nevertheless, as worldwide rates of adoption of herbicide-resistant crops and of glyphosate use have risen in recent years (Duke & Powles, 2009), restriction of glyphosate use has been enacted or proposed in several countries, although documented actions are few. In 2013, the Legislative Assembly of El Salvador voted a ban on the use of pesticides containing glyphosate (República de El Salvador, 2013). Sri Lanka is reported to have instituted a partial ban based on an increasing number of cases of chronic kidney disease among agricultural workers, but the ban was lifted after 2 months (ColomboPage, 2014). The reasons for such actions have included the development of resistance among weed species, as well as health concerns.

No limits for occupational exposure were identified by the Working Group.

1.3 Measurement and analysis

Several methods exist for the measurement of glyphosate and its major metabolite aminomethyl phosphonic acid (AMPA) in various media, including air, water, urine, and serum (Table 1.1). The methods largely involve derivatization with 9-fluorenylmethyl chloroformate (FMOC-Cl) to reach sufficient retention in chromatographic columns (Kuang *et al.*, 2011; Botero-Coy *et al.*, 2013). Chromatographic techniques that do not require derivatization and enzyme-linked immunosorbent assays (ELISA) are under development (Sanchís *et al.*, 2012).

Table 1.1 Methods for the analysis of glyphosate

Sample matrix	Assay procedure	Limit of detection	Reference
Water	HPLC/MS (with online solid-phase extraction)	0.08 µg/L	Lee *et al.* (2001)
	ELISA	0.05 µg/L	Abraxis (2005)
	LC-LC-FD	0.02 µg/L	Hidalgo *et al.* (2004)
	Post HPLC column derivatization and FD	6.0 µg/L	EPA (1992)
	UV visible spectrophotometer (at 435 ng)	1.1 µg/L	Jan *et al.* (2009)
Soil	LC–MS/MS with triple quadrupole	0.02 mg/kg	Botero-Coy *et al.* (2013)
Dust	GC-MS-MID	0.0007 mg/kg	Curwin *et al.* (2005)
Air	HPLC/MS with online solid-phase extraction	0.01 ng/m³	Chang *et al.* (2011)
Fruits and vegetables	HILIC/WAX with ESI-MS/MS	1.2 µg/kg	Chen *et al.* (2013)
Field crops (rice, maize and soybean)	LC–ESI-MS/MS	0.007–0.12 mg/kg	Botero-Coy *et al.* (2013b)
Plant vegetation	HPLC with single polymeric amino column	0.3 mg/kg	Nedelkoska & Low (2004)
Serum	LC–MS/MS	0.03 µg/mL 0.02 µg/mL (aminomethylphosphonic acid) 0.01 µg/mL (3-methylphosphinicopropionic acid)	Yoshioka *et al.* (2011)
Urine	HPLC with post-column reaction and FD	1 µg/L	Acquavella *et al.* (2004)
	ELISA	0.9 µg/L	Curwin *et al.* (2007)

ELISA, enzyme-linked immunosorbent assay; ESI-MS/MS, electrospray tandem mass spectrometry; FD, fluorescence detection; GC-MS-MID, gas chromatography-mass spectrometry in multiple ion detection mode; HILIC/WAX, hydrophilic interaction/weak anion-exchange liquid chromatography; HPLC/MS, high-performance liquid chromatography with mass spectrometry; HPLC, high-performance liquid chromatography; LC-ESI–MS/MS, liquid chromatography-electrospray–tandem mass spectrometry; LC–LC, coupled-column liquid chromatography; LC–MS/MS, liquid chromatography–tandem mass spectrometry

1.4 Occurrence and exposure

1.4.1 Exposure

(a) Occupational exposure

Studies related to occupational exposure to glyphosate have included farmers and tree nursery workers in the USA, forestry workers in Canada and Finland, and municipal weed-control workers in the United Kingdom (Centre de Toxicologie du Québec, 1988; Jauhiainen *et al.*, 1991; Lavy *et al.*, 1992; Acquavella *et al.*, 2004; Johnson *et al.*, 2005). Para-occupational exposures to glyphosate have also been measured in farming families (Acquavella *et al.*, 2004; Curwin *et al.*, 2007). These studies are summarized in Table 1.2.

(b) Community exposure

Glyphosate can be found in soil, air, surface water, and groundwater (EPA, 1993a). Once in the environment, glyphosate is adsorbed to soil and is broken down by soil microbes to AMPA (Borggaard & Gimsing, 2008). In surface water, glyphosate is not readily broken down by water or sunlight (EPA, 1993a). Despite extensive worldwide use, there are relatively few studies

Table 1.2 Occupational and para-occupational exposure to glyphosate

Industry, country, year	Job/process	Results	Comments/additional data	Reference
Forestry				
Canada, 1986		Arithmetic mean of air glyphosate concentrations:	Air concentrations of glyphosate were measured at the work sites of one crew (five workers) during ground spraying 268 urine samples were collected from 40 workers; glyphosate concentration was above the LOD (15 µg/L) in 14%	Centre de Toxicologie du Québec (1988)
	Signaller	Morning, 0.63 µg/m³ Afternoon, 2.25 µg/m³		
	Operator	Morning, 1.43 µg/m³ Afternoon, 6.49 µg/m³		
	Overseer	Morning, 0.84 µg/m³ Afternoon, 2.41 µg/m³		
	Mixer	Morning, 5.15 µg/m³ Afternoon, 5.48 µg/m³		
Finland, year NR	Workers performing silvicultural clearing (n = 5)	Range of air glyphosate concentrations, < 1.25–15.7 µg/m³ (mean, NR)	Clearing work was done with brush saws equipped with pressurized herbicide sprayers Air samples were taken from the workers' breathing zone (number of samples, NR) Urine samples were collected during the afternoons of the working week (number, NR) Glyphosate concentrations in urine were below the LOD (10 µg/L)	Jauhiainen *et al.* (1991)
USA, year NR	Workers in two tree nurseries (n = 14)	In dermal sampling, 1 of 78 dislodgeable residue samples were positive for glyphosate The body portions receiving the highest exposure were ankles and thighs	Dermal exposure was assessed with gauze patches attached to the clothing and hand rinsing Analysis of daily urine samples repeated over 12 weeks was negative for glyphosate	Lavy *et al.* (1992)
Weed control				
United Kingdom, year NR	Municipal weed control workers (n = 18)	Median, 16 mg/m³ in 85% of 21 personal air samples for workers spraying with mechanized all-terrain vehicle Median, 0.12 mg/m³ in 33% of 12 personal air samples collected from workers with backpack with lance applications	[The Working Group noted that the reported air concentrations were substantially higher than in other studies, but was unable to confirm whether the data were for glyphosate or total spray fluid] Dermal exposure was also measured, but reported as total spray fluid, rather than glyphosate	Johnson *et al.* (2005)

Case 3:16-md-02741-VC Document 654-2 Filed 10/28/17 Page 52 of 138

Table 1.2 (continued)

Industry, country, year	Job/process	Results	Comments/additional data	Reference
Farming				
USA, 2001	Occupational and para-occupational exposure of 24 farm families (24 fathers, 24 mothers and 65 children). Comparison group: 25 non-farm families (23 fathers, 24 mothers and 51 children)	Geometric mean (range) of glyphosate concentrations in urine: Non-farm fathers, 1.4 µg/L (0.13–5.4) Farm fathers, 1.9 µg/L (0.02–18) Non-farm mothers, 1.2 µg/L (0.06–5.0) Farm mothers, 1.5 µg/L (0.10–11) Non-farm children, 2.7 µg/L (0.10–9.4) Farm children, 2.0 µg/L (0.02–18)	Frequency of glyphosate detection ranged from 66% to 88% of samples (observed concentrations below the LOD were not censored). Detection frequency and geometric mean concentration were not significantly different between farm and non-farm families (observed concentrations below the LOD were not censored)	Curwin *et al.* (2007)
USA, year NR	Occupational and para-occupational exposures of 48 farmers, their spouses, and 79 children	Geometric mean (range) of glyphosate concentration in urine on day of application: Farmers, 3.2 µg/L (< 1 to 233 µg/L) Spouses, NR (< 1 to 3 µg/L) Children, NR (< 1 to 29 µg/L)	24-hour composite urine samples for each family member the day before, the day of, and for 3 days after a glyphosate application. Glyphosate was detected in 60% of farmers' samples, 4% of spouses' samples and 12% of children's samples the day of spraying and in 27% of farmers' samples, 2% of spouses' samples and 5% of children's samples 3 days after	Acquavella *et al.* (2004)

LOD, limit of detection; ND, not detected; NR, not reported

Case 3:16-md-02741-VC Document 654-2 Filed 10/28/17 Page 53 of 138

on the environmental occurrence of glyphosate (Kolpin *et al.*, 2006).

(i) Air

Very few studies of glyphosate in air were available to the Working Group. Air and rainwater samples were collected during two growing seasons in agricultural areas in Indiana, Mississippi, and Iowa, USA (Chang *et al.*, 2011). The frequency of glyphosate detection ranged from 60% to 100% in air and rain samples, and concentrations ranged from < 0.01 to 9.1 ng/m^3 in air samples and from < 0.1 to 2.5 µg/L in rainwater samples. Atmospheric deposition was measured at three sites in Alberta, Canada. Rainfall and particulate matter were collected as total deposition at 7-day intervals throughout the growing season. Glyphosate deposition rates ranged from < 0.01 to 1.51 µg/m^2 per day (Humphries *et al.*, 2005).

No data were available to the Working Group regarding glyphosate concentrations in indoor air.

(ii) Water

Glyphosate in the soil can leach into groundwater, although the rate of leaching is believed to be low (Borggaard & Gimsing, 2008; Simonsen *et al.*, 2008). It can also reach surface waters by direct emission, atmospheric deposition, and by adsorption to soil particles suspended in runoff water (EPA, 1993a; Humphries *et al.*, 2005). Table 1.3 summarizes data on concentrations of glyphosate or AMPA in surface water and groundwater.

(iii) Residues in food and dietary intake

Glyphosate residues have been measured in cereals, fruits, and vegetables (Table 1.4). Residues were detected in 0.04% of 74 305 samples of fruits, vegetables, and cereals tested from 27 member states of the European Union, and from Norway, and Iceland in 2007 (EFSA, 2009). In cereals, residues were detected in 50% of samples tested in Denmark in 1998–1999, and in 9.5% of samples tested from member states of the European Union, and from Norway and Iceland in 2007 (Granby & Vahl, 2001; EFSA, 2009). In the United Kingdom, food sampling for glyphosate residues has concentrated mainly on cereals, including bread and flour. Glyphosate has been detected regularly and usually below the reporting limit (Pesticide Residues Committee, 2007, 2008, 2009, 2010). Six out of eight samples of tofu made from Brazilian soy contained glyphosate, with the highest level registered being 1.1 mg/kg (Pesticide Residues Committee, 2007).

(iv) Household exposure

In a survey of 246 California households, 14% were found to possess at least one product containing glyphosate (Guha *et al.*, 2013).

(v) Biological markers

Glyphosate concentrations in urine were analysed in urban populations in Europe, and in a rural population living near areas sprayed for drug eradication in Colombia (MLHB, 2013; Varona *et al.*, 2009). Glyphosate concentrations in Colombia were considerably higher than in Europe, with means of 7.6 ng/L and 0.02 µg/L, respectively (Table 1.5). In a study in Canada, glyphosate concentrations in serum ranged from undetectable to 93.6 ng/mL in non-pregnant women (n = 39), and were undetectable in serum of pregnant women (n = 30) and fetal cord serum (Aris & Leblanc, 2011).

1.4.2 Exposure assessment

Exposure assessment methods in epidemiological studies on glyphosate and cancer are discussed in Section 2.0 of the *Monograph* on Malathion, in the present volume.

Table 1.3 Concentration of glyphosate and AMPA in water

Country, year of sampling	Number of samples/setting	Results	Comments/additional data	Reference
USA, 2002	51 streams/agricultural areas (154 samples)	Maximum glyphosate concentration, 5.1 µg/L Maximum AMPA concentration, 3.67 µg/L	The samples were taken following pre- and post-emergence application and during harvest season Glyphosate detected in 36% of samples; AMPA detected in 69% of samples	Battaglin *et al.*, (2005)
USA, 2002	10 wastewater treatment plants and two reference streams (40 samples)	Glyphosate, range ≤ 0.1–2 µg/L AMPA, range ≤ 0.1–4 µg/L	AMPA was detected more frequently (67.5%) than glyphosate (17.5%)	Kolpin *et al.* (2006)
Canada, 2002	3 wetlands and 10 agricultural streams (74 samples)	Range, < 0.02–6.08 µg/L	Glyphosate was detected in most of the wetlands and streams (22% of samples)	Humphries *et al.* (2005)
Colombia, year NR	5 areas near crops and coca eradication (24 samples)	Maximum concentration, 30.1 µg/L (minimum and mean, NR)	Glyphosate detected in 8% of samples (MDL, 25 µg/L)	Solomon *et al.*, (2007)
Denmark, 2010–2012	4 agricultural sites (450 samples)	Range, < 0.1–31.0 µg/L	Glyphosate detected in 23% of samples; AMPA detected in 25% of samples	Brüch *et al.* (2013)

AMPA, aminomethylphosphonic acid; MDL, method detection limit; NR, data not reported

Case 3:16-md-02741-VC Document 654-2 Filed 10/28/17 Page 55 of 138

Case 3:16-md-02741-VC Document 654-2 Filed 10/28/17 Page 56 of 138

Table 1.4 Concentrations of glyphosate in food

Country, year	Type of food	Results	Comments/additional data	Reference
Denmark, 1998, 1999	Cereals	> 50% of samples had detectable residues Means: 0.08 mg/kg in 1999 and 0.11 mg/kg in 1998	49 samples of the 1998 harvest 46 samples of the 1999 harvest	Granby & Vahl (2001)
27 European Union member states, Norway and Iceland, 2007	350 different food commodities	0.04% of 2302 fruit, vegetable and cereal samples 9.5% of 409 cereal samples	74 305 total samples	EFSA (2009)
Australia, 2006	Composite sample of foods consumed in 24 hours	75% of samples had detectable residues Mean, 0.08 mg/kg Range, < 0.005 to 0.5 mg/kg	20 total samples from 43 pregnant women	McQueen *et al.* (2012)

Table 1.5 Concentrations of glyphosate and AMPA in urine and serum in the general population

Country, period	Subjects	Results	Comments/additional data	Reference
Urine				
18 European countries, 2013	162 individuals	Arithmetic mean of glyphosate concentration: 0.21 µg/L (maximum, 1.56 µg/L) Arithmetic mean of AMPA concentration: 0.19 µg/L (maximum, 2.63 µg/L)	44% of samples had quantifiable levels of glyphosate and 36% had quantifiable levels of AMPA	MLHB (2013)
Colombia, 2005–2006	112 residents of areas sprayed for drug eradication	Arithmetic mean (range) of glyphosate concentration: 7.6 µg/L (ND–130 µg/L) Arithmetic mean (range) of AMPA concentration: 1.6 µg/L (ND–56 µg/L)	40% of samples had detectable levels of glyphosate and 4% had detectable levels of AMPA (LODs, 0.5 and 1.0 µg/L, respectively) Urinary glyphosate was associated with use in agriculture	Varona *et al.* (2009)
Serum				
Canada, NR	30 pregnant women and 39 non-pregnant women	ND in serum of pregnant women or cord serum; Arithmetic mean, 73.6 µg/L, (range, ND–93.6 µg/L) in non-pregnant women	No subject had worked or lived with a spouse working in contact with pesticides LOD, 15 µg/L	Aris & Leblanc (2011)

AMPA, aminomethylphosphonic acid; LOD, limit of detection; ND, not detected; NR, not reported

2. Cancer in Humans

2.0 General discussion of epidemiological studies

A general discussion of the epidemiological studies on agents considered in Volume 112 of the *IARC Monographs* is presented in Section 2.0 of the *Monograph* on Malathion.

2.1 Cohort studies

See Table 2.1

The Agricultural Health Study (AHS), a large prospective cohort study conducted in Iowa and North Carolina in the USA, is the only cohort study to date to have published findings on exposure to glyphosate and the risk of cancer at many different sites (Alavanja *et al.*, 1996; NIH, 2015) (see Section 2.0 of the *Monograph* on Malathion, in the present volume, for a detailed description of this study).

The enrolment questionnaire from the AHS sought information on the use of 50 pesticides (ever or never exposure), crops grown and livestock raised, personal protective equipment used, pesticide application methods used, other agricultural activities and exposures, nonfarm occupational exposures, and several lifestyle, medical, and dietary variables. The duration (years) and frequency (days per year) of use was investigated for 22 of the 50 pesticides in the enrolment questionnaire. [Blair *et al.* (2011) assessed the possible impact of misclassification of occupational pesticide exposure on relative risks, demonstrating that nondifferential exposure misclassification biases relative risk estimates towards the null in the AHS and tends to decrease the study power.]

The first report of cancer incidence associated with pesticide use in the AHS cohort considered cancer of the prostate (Alavanja *et al.*, 2003). Risk estimates for exposure to glyphosate were not presented, but no significant exposure–response association with cancer of the prostate was found. In an updated analysis of the AHS (1993 to 2001), De Roos *et al.* (2005a) (see below) also found no association between exposure to glyphosate and cancer of the prostate (relative risk, RR, 1.1; 95% CI, 0.9–1.3) and no exposure–response trend (*P* value for trend = 0.69).

De Roos *et al.* (2005a) also evaluated associations between exposure to glyphosate and the incidence of cancer at several other sites. The prevalence of ever-use of glyphosate was 75.5% (> 97% of users were men). In this analysis, exposure to glyphosate was defined as: (a) ever personally mixed or applied products containing glyphosate; (b) cumulative lifetime days of use, or "cumulative exposure days" (years of use × days/year); and (c) intensity-weighted cumulative exposure days (years of use × days/year × estimated intensity level). Poisson regression was used to estimate exposure–response relations between exposure to glyphosate and incidence of all cancers combined, and incidence of 12 cancer types: lung, melanoma, multiple myeloma, and non-Hodgkin lymphoma (see Table 2.1) as well as oral cavity, colon, rectum, pancreas, kidney, bladder, prostate, and leukaemia (results not tabulated). Exposure to glyphosate was not associated with all cancers combined (RR, 1.0; 95% CI, 0.9–1.2; 2088 cases). For multiple myeloma, the relative risk was 1.1 (95% CI, 0.5–2.4; 32 cases) when adjusted for age, but was 2.6 (95% CI, 0.7–9.4) when adjusted for multiple confounders (age, smoking, other pesticides, alcohol consumption, family history of cancer, and education); in analyses by cumulative exposure-days and intensity-weighted exposure-days, the relative risks were around 2.0 in the highest tertiles. Furthermore, the association between multiple myeloma and exposure to glyphosate only appeared within the subgroup for which complete data were available on all the covariates; even without any adjustment, the risk of multiple myeloma associated with glyphosate use was increased by twofold among the smaller subgroup with available covariate data

Case 3:16-md-02741-VC Document 654-2 Filed 10/28/17 Page 58 of 138

Table 2.1 Cohort studies of cancer and exposure to glyphosate

Reference, study location, enrolment period/follow-up, study-design	Population size, description, exposure assessment method	Organ site (ICD code)	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
De Roos *et al.* (2005a) Iowa and North Carolina, USA 1993–2001	54 315 (after exclusions, from a total cohort of 57 311) licensed pesticide applicators Exposure assessment method: questionnaire; semi-quantitative assessment from self-administered questionnaire	Lung	Ever use	147	0.9 (0.6–1.3)	Age, smoking, other pesticides, alcohol consumption, family history of cancer, education	AHS Cancer sites investigated: lung, melanoma, multiple myeloma and NHL (results tabulated) as well as oral cavity, colon, rectum, pancreas, kidney, bladder, prostate and leukaemia (results not tabulated) [Strengths: large cohort; specific assessment of glyphosate; semiquantitative exposure assessment. Limitations: risk estimates based on self-reported exposure; limited to licensed applicators; potential exposure to multiple pesticides]
			Cumulative exposure days:				
			1–20	40	1 (ref.)		
			21–56	26	0.9 (0.5–1.5)		
			57–2678	26	0.7 (0.4–1.2)		
			Trend-test *P* value: 0.21				
		Melanoma	Ever use	75	1.6 (0.8–3)		
			1–20	23	1 (ref.)		
			21–56	20	1.2 (0.7–2.3)		
			57–2678	14	0.9 (0.5–1.8)		
			Trend-test *P* value: 0.77				
		Multiple myeloma	Ever use	32	1.1 (0.5–2.4)	Age only (results in this row only)	
			Ever use	32	2.6 (0.7–9.4)		
			1–20	8	1 (ref.)		
			21–56	5	1.1 (0.4–3.5)		
			Trend-test *P* value: 0.27				
		NHL	Ever use	92	1.1 (0.7–1.9)		
			1–20	29	1 (ref.)		
			21–56	15	0.7 (0.4–1.4)		
			57–2678	17	0.9 (0.5–1.6)		
			Trend-test *P* value: 0.73				

Table 2.1 (continued)

Reference, study location, enrolment period/follow-up, study-design	Population size, description, exposure assessment method	Organ site (ICD code)	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Flower *et al.* (2004) Iowa and North Carolina, USA Enrolment, 1993–1997; follow-up, 1975–1998	21 375; children (aged < 19 years) of licensed pesticide applicators in Iowa (n = 17 357) and North Carolina (n = 4018) Exposure assessment method: questionnaire	Childhood cancer	Maternal use of glyphosate (ever) Paternal use of glyphosate (prenatal)	13 6	0.61 (0.32–1.16) 0.84 (0.35–2.34)	Child's age at enrolment	AHS Glyphosate results relate to the Iowa participants only [Strengths: Large cohort; specific assessment of glyphosate. Limitations: based on self-reported exposure; potential exposure to multiple pesticides; limited power for glyphosate exposure]
Engel *et al.* (2005) Iowa and North Carolina, USA Enrolment, 1993–1997 follow-up to 2000	30 454 wives of licensed pesticide applicators with no history of breast cancer at enrolment Exposure assessment method: questionnaire	Breast	Direct exposure to glyphosate Husband's use of glyphosate	82 109	0.9 (0.7–1.1) 1.3 (0.8–1.9)	Age, race, state	AHS [Strengths: large cohort; specific assessment of glyphosate. Limitations: based on self-reported exposure; limited to licensed applicators; potential exposure to multiple pesticides]
Lee *et al.* (2007) Iowa and North Carolina, USA Enrolment, 1993–1997; follow-up to 2002	56 813 licensed pesticide applicators Exposure assessment method: questionnaire	Colorectum Colon Rectum	Exposed to glyphosate Exposed to glyphosate Exposed to glyphosate	225 151 74	1.2 (0.9–1.6)	Age, smoking, state, total days of any pesticide application	AHS [Strengths: large cohort. Limitations: based on self-reported exposure, limited to licensed applicators, potential

Case 3:16-md-02741-VC Document 654-2 Filed 10/28/17 Page 59 of 138

Table 2.1 (continued)

Reference, study location, enrolment period/follow-up, study-design	Population size, description, exposure assessment method	Organ site (ICD code)	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Andreotti *et al.* (2009) Iowa and North Carolina, USA Enrolment, 1993–1997; follow-up to 2004 Nested case–control study	Cases: 93 (response rate, NR); identified from population-based state-cancer registries. Incident cases diagnosed between enrolment and 31 December 2004 (> 9 years follow-up) included in the analysis. Participants with any type of prevalent cancer at enrolment were excluded. Vital status was obtained from the state death registries and the National Death Index. Participants who left North Carolina or Iowa were not subsequently followed for cancer occurrence. Controls: 82 503 (response rate, NR); cancer-free participants enrolled in the cohort Exposure assessment method: questionnaire providing detailed pesticide use, demographic and lifestyle information. Ever-use of 24 pesticides and intensity-weighted lifetime days [(lifetime exposure days) × (exposure intensity score)] of 13 pesticides was assessed	Pancreas (C25.0–C25.9)	Ever exposure to glyphosate Low (< 185 days) High (≥ 185 days) Trend-test *P* value: 0.85	55 29 19	1.1 (0.6–1.7)	Age, smoking, diabetes	AHS [Strengths: large cohort. Limitations: based on self-reported exposure; limited to licensed applicators; potential exposure to multiple pesticides]

AHS, Agricultural Health Study; NHL, non-Hodgkin lymphoma; NR, not reported

Case 3:16-md-02741-VC Document 654-2 Filed 10/28/17 Page 60 of 138

(De Roos *et al.*, 2005b). [The study had limited power for the analysis of multiple myeloma; there were missing data on covariates when multiple adjustments were done, limiting the interpretation of the findings.] A re-analysis of these data conducted by Sorahan (2015) confirmed that the excess risk of multiple myeloma was present only in the subset with no missing information (of 22 cases in the restricted data set). In a subsequent cross-sectional analysis of 678 male participants from the same cohort, Landgren *et al.* (2009) did not find an association between exposure to glyphosate and risk of monoclonal gammopathy of undetermined significance (MGUS), a premalignant plasma disorder that often precedes multiple myeloma (odds ratio, OR, 0.5; 95% CI, 0.2–1.0; 27 exposed cases).

Flower *et al.* (2004) reported the results of the analyses of risk of childhood cancer associated with pesticide application by parents in the AHS. The analyses for glyphosate were conducted among 17 357 children of Iowa pesticide applicators from the AHS. Parents provided data via questionnaires (1993–1997) and the cancer follow-up (retrospectively and prospectively) was done through the state cancer registries. Fifty incident childhood cancers were identified (1975–1998; age, 0–19 years). For all the children of the pesticide applicators, risk was increased for all childhood cancers combined, for all lymphomas combined, and for Hodgkin lymphoma, compared with the general population. The odds ratio for use of glyphosate and risk of childhood cancer was 0.61 (95% CI, 0.32–1.16; 13 exposed cases) for maternal use and 0.84 (95% CI, 0.35–2.34; 6 exposed cases) for paternal use. [The Working Group noted that this analysis had limited power to study a rare disease such as childhood cancer.]

Engel *et al.* (2005) reported on incidence of cancer of the breast among farmers' wives in the AHS cohort, which included 30 454 women with no history of cancer of the breast before enrolment in 1993–1997. Information on pesticide use and other factors was obtained at enrolment by self-administered questionnaire from the women and their husbands. A total of 309 incident cases of cancer of the breast were identified until 2000. There was no difference in incidence of cancer of the breast for women who reported ever applying pesticides compared with the general population. The relative risk for cancer of the breast among women who had personally used glyphosate was 0.9 (95% CI, 0.7–1.1; 82 cases) and 1.3 (95% CI, 0.8–1.9; 109 cases) among women who never used pesticides but whose husband had used glyphosate. [No information on duration of glyphosate use by the husband was presented.] Results for glyphosate were not further stratified by menopausal status.

Lee *et al.* (2007) investigated the relationship between exposure to agricultural pesticides and incidence of cancer of the colorectum in the AHS. A total of 56 813 pesticide applicators with no prior history of cancer of the colorectum were included in this analysis, and 305 incident cancers of the colorectum (colon, 212; rectum, 93) were diagnosed during the study period, 1993–2002. Most of the 50 pesticides studied were not associated with risk of cancer of the colorectum, and the relative risks with exposure to glyphosate were 1.2 (95% CI, 0.9–1.6), 1.0 (95% CI, 0.7–1.5), and 1.6 (95% CI, 0.9–2.9) for cancers of the colorectum, colon, and rectum, respectively.

Andreotti *et al.* (2009) examined associations between the use of pesticides and cancer of the pancreas using a case–control analysis nested in the AHS. This analysis included 93 incident cases of cancer of the pancreas (64 applicators, 29 spouses) and 82 503 cancer-free controls who completed the enrolment questionnaire. Ever-use of 24 pesticides and intensity-weighted lifetime days [(lifetime exposure days) × (exposure intensity score)] of 13 pesticides were assessed. Risk estimates were calculated controlling for age, smoking, and diabetes. The odds ratio for ever- versus never-exposure to glyphosate was

1.1 (95% CI, 0.6–1.7; 55 exposed cases), while the odds ratio for the highest category of level of intensity-weighted lifetime days was 1.2 (95% CI, 0.6–2.6; 19 exposed cases).

Dennis *et al.* (2010) reported that exposure to glyphosate was not associated with cutaneous melanoma within the AHS. [The authors did not report a risk estimate.]

2.2 Case–control studies on non-Hodgkin lymphoma, multiple myeloma, and leukaemia

2.2.1 Non-Hodgkin lymphoma

See Table 2.2

(a) Case–control studies in the midwest USA

Cantor *et al.* (1992) conducted a case–control study of incident non-Hodgkin lymphoma (NHL) among males in Iowa and Minnesota, USA (see the *Monograph* on Malathion, Section 2.0, for a detailed description of this study). A total of 622 white men and 1245 population-based controls were interviewed in person. The association with farming occupation and specific agricultural exposures were evaluated. When compared with non-farmers, the odds ratios for NHL were 1.2 (95% CI, 1.0–1.5) for men who had ever farmed, and 1.1 (95% CI, 0.7–1.9; 26 exposed cases; adjusted for vital status, age, state, cigarette smoking status, family history of lymphohaematopoietic cancer, high-risk occupations, and high-risk exposures) for ever handling glyphosate. [There was low power to assess the risk of NHL associated with exposure to glyphosate. There was no adjustment for other pesticides. These data were included in the pooled analysis by De Roos *et al.* (2003).]

Brown *et al.* (1993) reported the results of a study to evaluate the association between multiple myeloma and agricultural risk factors in the midwest USA (see the *Monograph* on Malathion, Section 2.0, for a detailed description of this study). A population-based case–control study of 173 white men with multiple myeloma and 650 controls was conducted in Iowa, USA, an area with a large farming population. A non-significantly elevated risk of multiple myeloma was seen among farmers compared with never-farmers. The odds ratio related to exposure to glyphosate was 1.7 (95% CI, 0.8–3.6; 11 exposed cases). [This study had limited power to assess the association between multiple myeloma and exposure to glyphosate. Multiple myeloma is now considered to be a subtype of NHL.]

De Roos *et al.* (2003) used pooled data from three case–control studies of NHL conducted in the 1980s in Nebraska (Zahm *et al.*, 1990), Kansas (Hoar *et al.*, 1986), and in Iowa and Minnesota (Cantor *et al.*, 1992) (see the *Monograph* on Malathion, Section 2.0, for a detailed description of these studies) to examine pesticide exposures in farming as risk factors for NHL in men. The study population included 870 cases and 2569 controls; 650 cases and 1933 controls were included for the analysis of 47 pesticides controlling for potential confounding by other pesticides. Both logistic regression and hierarchical regression (adjusted estimates were based on prior distributions for the pesticide effects, which provides more conservative estimates than logistic regression) were used in data analysis, and all models were essentially adjusted for age, study site, and other pesticides. Reported use of glyphosate as well as several individual pesticides was associated with increased incidence of NHL. Based on 36 cases exposed, the odds ratios for the association between exposure to glyphosate and NHL were 2.1 (95% CI, 1.1–4.0) in the logistic regression analyses and 1.6 (95% CI, 0.9–2.8) in the hierarchical regression analysis. [The numbers of cases and controls were lower than those in the pooled analysis by Waddell *et al.* (2001) because only subjects with no missing data on pesticides were included. The strengths of this study when compared with other studies are that it was large,

Table 2.2 Case–control studies of leukaemia and lymphoma and exposure to glyphosate

Reference, location, enrolment period	Population size, description, exposure assessment method	Organ site (ICD code)	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
USA							
Brown *et al.* (1990) Iowa and Minnesota, USA 1981–1983	Cases: 578 (340 living, 238 deceased) (response rate, 86%); cancer registry or hospital records Controls: 1245 (820 living, 425 deceased) (response rate, 77–79%); random-digit dialling for those aged < 65 years and Medicare for those aged ≥ 65 years Exposure assessment method: questionnaire	Leukaemia	Any glyphosate	15	0.9 (0.5–1.6)	Age, vital status, state, tobacco use, family history lymphopoietic cancer, high-risk occupations, high risk exposures	[Strengths: large population based study in a farming area. Limitations: not controlled for exposure to other pesticides. Limited power for glyphosate exposure]
Cantor *et al.* (1992) Iowa and Minnesota, USA 1980–1982	Cases: 622 (response rate, 89.0%); Iowa health registry records and Minnesota hospital and pathology records Controls: 1245 (response rate, 76–79%); population-based; no cancer of the lympho-haematopoietic system; frequency-matched to cases by age (5-year group), vital status, state. Random-digit dialling (aged < 65 years); Medicare records (aged ≥ 65 years); state death certificate files (deceased subjects) Exposure assessment method: questionnaire; in-person interview	NHL	Ever handled glyphosate	26	1.1 (0.7–1.9)	Age, vital status, state, smoking status, family history lymphopoietic cancer, high-risk occupations, high-risk exposures	Data subsequentially pooled in De Roos *et al.* (2003); white men only [Strengths: large population-based study in farming areas. Limitations: not controlled for exposure to other pesticides. Limited power for glyphosate exposure]

Case 3:16-md-02741-VC Document 654-2 Filed 10/28/17 Page 63 of 138

Case 3:16-md-02741-VC Document 654-2 Filed 10/28/17 Page 64 of 138

Table 2.2 (continued)

Reference, location, enrolment period	Population size, description, exposure assessment method	Organ site (ICD code)	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Brown *et al.* (1993) Iowa, USA 1981–1984	Cases: 173 (response rate, 84%); Iowa health registry Controls: 650 (response rate, 78%); Random-digit dialling (aged < 65 years) and Medicare (aged > 65 years) Exposure assessment method: questionnaire	Multiple myeloma	Any glyphosate	11	1.7 (0.8–3.6)	Age, vital status	[Strengths: population-based study. Areas with high prevalence of farming. Limitations: limited power for glyphosate exposure]
De Roos *et al.* (2003) Nebraska, Iowa, Minnesota, Kansas, USA 1979–1986	Cases: 650 (response rate, 74.7%); cancer registries and hospital records Controls: 1933 (response rate, 75.2%); random-digit dialling, Medicare, state mortality files Exposure assessment method: questionnaire; interview (direct or next-of-kin)	NHL	Any glyphosate exposure	36	2.1 (1.1–4)	Age, study area, other pesticides	Both logistic regression and hierarchical regression were used in data analysis, the latter providing more conservative estimates [Strengths: increased power when compared with other studies, population-based, and conducted in farming areas. Advanced analytical methods to account for multiple exposures] Included participants from Cantor *et al.* (1992), Zahm *et al.* (1990), Hoar *et al.* (1986), and Brown *et al.* (1990)

Table 2.2 (continued)

Reference, location, enrolment period	Population size, description, exposure assessment method	Organ site (ICD code)	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Lee *et al.* (2004a) Iowa, Minnesota and Nebraska, USA 1980–1986	Cases: 872 (response rate, NR); diagnosed with NHL from 1980 to 1986 Controls: 2381 (response rate, NR); frequency-matched controls Exposure assessment method: questionnaire; information on use of pesticides and history of asthma was based on interviews	NHL	Exposed to glyphosate – non-asthmatics Exposed to glyphosate – asthmatics	53 6	1.4 (0.98–2.1) 1.2 (0.4–3.3)	Age, vital status, state	177 participants (45 NHL cases, 132 controls) reported having been told by their doctor that they had asthma
Canada							
McDuffie *et al.* (2001) Canada 1991–1994	Cases: 517 (response rate, 67.1%), from cancer registries and hospitals Controls: 1506 (response rate, 48%); random sample from health insurance and voting records Exposure assessment method: questionnaire, some administered by telephone, some by post	NHL	Exposed to glyphosate Unexposed > 0 and ≤ 2 days > 2 days	51 464 28 23	1.2 (0.83–1.74) 1 1.0 (0.63–1.57) 2.12 (1.2–3.73)	Age, province of residence	Cross-Canada study [Strengths: large population based study. Limitations: no quantitative exposure data. Exposure assessment by questionnaire. Relatively low participation]

Case 3:16-md-02741-VC Document 654-2 Filed 10/28/17 Page 65 of 138

Case 3:16-md-02741-VC Document 654-2 Filed 10/28/17 Page 66 of 138

Table 2.2 (continued)

Reference, location, enrolment period	Population size, description, exposure assessment method	Organ site (ICD code)	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Karunanayake *et al.* (2012) Six provinces in Canada (Quebec, Ontario, Manitoba, Saskatchewan, Alberta, and British Columbia) 1991–1994	Incident cases: 316 (response rate, 68.4%); men aged ≥ 19 years; ascertained from provincial cancer registries, except in Quebec (hospital ascertainment) Controls: 1506 (response rate, 48%); matched by age ± 2 years to be comparable with the age distribution of the entire case group (HL, NHL, MM, and STS) within each province of residence. Potential controls (men aged ≥ 19 years) selected at random within age constraints from the provincial health insurance records (Alberta, Saskatchewan, Manitoba, Quebec), computerized telephone listings (Ontario), or voters' lists (British Columbia) Exposure assessment method: questionnaire; stage 1 used a self-administered postal questionnaire; and in stage 2 detailed pesticide exposure information was collected by telephone interview	HL (ICDO2 included nodular sclerosis (M9656/3; M9663/3; M9664/3; M9665/3; M9666/3; M9667/3), lymphocytic predominance (M9651/3; M9657/3; M9658/3; M9659/3), mixed cellularity (M9652/3), lymphocytic depletion (M9653/3; M9654/3), miscellaneous (other M9650-M9669 codes for HL)	Glyphosate-based formulation	38	1.14 (0.74–1.76)	Age group, province of residence	Cross Canada study Based on the statistical analysis of pilot study data, it was decided that the most efficient definition of pesticide exposure was a cumulative exposure ≥ 10 hours/year to any combination of pesticides. This discriminated (a) between incidental, bystander, and environmental exposure vs more intensive exposure, and (b) between cases and controls [Strengths: large study. Limitations: low response rates]
			Glyphosate-based formulation	38	0.99 (0.62–1.56)	Age group, province of residence, medical history	

Table 2.2 (continued)

Reference, location, enrolment period	Population size, description, exposure assessment method	Organ site (ICD code)	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Kachuri *et al.* (2013) Six Canadian provinces (British Columbia, Alberta, Saskatchewan, Manitoba, Ontario and Quebec) 1991–1994	Cases: 342 (response rate, 58%); men aged ≥ 19 years diagnosed between 1991 and 1994 were ascertained from provincial cancer registries except in Quebec, where ascertained from hospitals Controls: 1357 (response rate, 48%); men aged ≥ 19 years selected randomly using provincial health insurance records, random digit dialling, or voters' lists, frequency-matched to cases by age (±2 years) and province of residence Exposure assessment method: questionnaire	Multiple myeloma	Glyphosate use	32	1.19 (0.76–1.87)	Age, province of residence, use of a proxy respondent, smoking status, medical variables, family history of cancer	Cross-Canada study [Strengths: population-based case–control study. Limitations: relatively low response rates]
			Use of glyphosate (> 0 and ≤ 2 days per year)	15	0.72 (0.39–1.32)		
			Use of glyphosate (> 2 days per year)	12	2.04 (0.98–4.23)		
Sweden							
Nordström *et al.* (1998) Sweden 1987–1992	Cases: 111 (response rate, 91%); 121 HCL cases in men identified from Swedish cancer registry Controls: 400 (response rate, 83%); 484 (four controls/case) matched for age and county; national population registry Exposure assessment method: questionnaire; considered exposed if minimum exposure of 1 working day (8 h) and an induction period of at least 1 year	HCL	Exposed to glyphosate	4	3.1 (0.8–12)	Age	Overlaps with Hardell *et al.* (2002). HCL is a subtype of NHL [Strengths: population-based case–control study. Limitations: Limited power. There was no adjustment for other exposures]

Case 3:16-md-02741-VC Document 654-2 Filed 10/28/17 Page 67 of 138

Table 2.2 (continued)

Reference, location, enrolment period	Population size, description, exposure assessment method	Organ site (ICD code)	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Hardell & Eriksson (1999) Northern and middle Sweden 1987–1990	Cases: 404 (192 deceased) (response rate, 91%); regional cancer registries Controls: 741 (response rate, 84%); live controls matched for age and county were recruited from the national population registry, and deceased cases matched for age and year of death were identified from the national registry for causes of death Exposure assessment method: questionnaire	NHL (ICD-9 200 and 202)	Ever glyphosate – univariate Ever glyphosate – multivariate	4 NR	2.3 (0.4–13) 5.8 (0.6–54)	Not specified in the multivariable analysis	Overlaps with Hardell *et al.* (2002) [Strengths: population-based study. Limitations: few subjects were exposed to glyphosate and the study had limited power. Analyses were "multivariate" but covariates were not specified]
Hardell *et al.* (2002) Sweden; four Northern counties and three counties in mid Sweden 1987–1992	Cases: 515 (response rate, 91% in both studies); Swedish cancer registry Controls: 1141 (response rates, 84% and 83%%); national population registry Exposure assessment method: questionnaire	NHL and HCL	Ever glyphosate exposure (univariate) Ever glyphosate exposure (multivariate)	8 8	3.04 (1.08–8.5) 1.85 (0.55–6.2)	Age, county, study site, vital status, other pesticides in the multivariate analysis	Overlaps with Nordström *et al.* (1998) and Hardell & Eriksson (1999), [Strengths: large population-based study. Limitations: limited power for glyphosate exposure]

Case 3:16-md-02741-VC Document 654-2 Filed 10/28/17 Page 68 of 138

Table 2.2 (continued)

Reference, location, enrolment period	Population size, description, exposure assessment method	Organ site (ICD code)	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Eriksson *et al.* (2008) Sweden. Four health service areas (Lund, Linkoping, Orebro and Umea) 1999–2002	Cases: 910 (response rate, 91%); incident NHL cases were enrolled from university hospitals Controls: 1016 (response rate, 92%); national population registry Exposure assessment method: questionnaire	NHL	Any glyphosate	29	2.02 (1.1–3.71)	Age, sex, year of enrolment	[Strengths: population-based case-control. Limitations: limited power for glyphosate] * Exposure to other pesticides (e.g. MPCA) controlled in the analysis
			Any glyphosate*	29	1.51 (0.77–2.94)		
			≤ 10 days per year use	12	1.69 (0.7–4.07)		
			> 10 days per year use	17	2.36 (1.04–5.37)		
		NHL	1–10 yrs	NR	1.11 (0.24–5.08)		
			> 10 yrs	NR	2.26 (1.16–4.4)		
		B-cell lymphoma	Exposure to glyphosate	NR	1.87 (0.998–3.51)		
		Lymphocytic lymphoma/B-CLL	Exposure to glyphosate	NR	3.35 (1.42–7.89)		
		Diffuse large B-cell lymphoma	Exposure to glyphosate	NR	1.22 (0.44–3.35)		
		Follicular, grade I–III	Exposure to glyphosate	NR	1.89 (0.62–5.79)		
		Other specified B-cell lymphoma	Exposure to glyphosate	NR	1.63 (0.53–4.96)		
		Unspecified B-cell lymphoma	Exposure to glyphosate	NR	1.47 (0.33–6.61)		
		T-cell lymphoma	Exposure to glyphosate	NR	2.29 (0.51–10.4)		
		Unspecified NHL	Exposure to glyphosate	NR	5.63 (1.44–22)		

Case 3:16-md-02741-VC Document 654-2 Filed 10/28/17 Page 69 of 138

Case 3:16-md-02741-VC Document 654-2 Filed 10/28/17 Page 70 of 138

Table 2.2 (continued)

Reference, location, enrolment period	Population size, description, exposure assessment method	Organ site (ICD code)	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Other studies in Europe							
Orsi *et al.* (2009) France 2000–2004	Cases: 491 (response rate, 95.7%); cases (244 NHL; 87 HL; 104 LPSs; 56 MM) were recruited from main hospitals of the French cities of Brest, Caen, Nantes, Lille, Toulouse and Bordeaux, aged 20–75 years; ALL cases excluded Controls: 456 (response rate, 91.2%); matched on age and sex, recruited in the same hospitals as the cases, mainly in orthopaedic and rheumatological departments and residing in the hospital's catchment area Exposure assessment method: questionnaire	NHL	Any glyphosate exposure	12	1.0 (0.5–2.2)	Age, centre, socioeconomic category (blue/ white collar)	[Limitations: limited power for glyphosate]
		HL	Any exposure to glyphosate	6	1.7 (0.6–5)		
		LPS	Any exposure to glyphosate	4	0.6 (0.2–2.1)		
		MM	Any exposure to glyphosate	5	2.4 (0.8–7.3)		
		All lymphoid neoplasms	Any exposure to glyphosate	27	1.2 (0.6–2.1)		
		NHL, diffuse large cell lymphoma	Occupational use of glyphosate	5	1.0 (0.3–2.7)		
		NHL, follicular lymphoma	Occupational exposure to glyphosate	3	1.4 (0.4–5.2)		
		LPS/CLL	Occupational exposure to glyphosate	2	0.4 (0.1–1.8)		
		LPS/HCL	Occupational exposure to glyphosate	2	1.8 (0.3–9.3)		

Table 2.2 (continued)

Reference, location, enrolment period	Population size, description, exposure assessment method	Organ site (ICD code)	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Cocco *et al.* (2013) Czech Republic, France, Germany, Italy, Ireland and Spain 1998–2004	Cases: 2348 (response rate, 88%); cases were all consecutive adult patients first diagnosed with lymphoma during the study period, resident in the referral area of the participating centres Controls: 2462 (response rate, 81% hospital; 52% population); controls from Germany and Italy were randomly selected by sampling from the general population and matched to cases on sex, 5-year age-group, and residence area. The rest of the centres used matched hospital controls, excluding diagnoses of cancer, infectious diseases and immunodeficiency diseases Exposure assessment method: questionnaire; support of a crop-exposure matrix to supplement the available information, industrial hygienists and occupational experts in each participating centre reviewed the general questionnaires and job modules to assess exposure to pesticides	B-cell lymphoma	Occupational exposure to glyphosate	4	3.1 (0.6–17.1)	Age, sex, education, centre	EPILYMPH case-control study in six European countries

ALL, acute lymphocytic leukaemia; B-CLL, chronic lymphocytic leukaemia; CLL, chronic lymphocytic leukaemia; HCL, hairy cell leukaemia; HL, Hodgkin lymphoma; LPS, lymphoproliferative syndrome; MCPA, 2-methyl-4-chlorophenoxyacetic acid; MM, multiple myeloma; NHL, non-Hodgkin lymphoma; NR, not reported; ref., reference; STS, soft tissue sarcoma

Case 3:16-md-02741-VC Document 654-2 Filed 10/28/17 Page 71 of 138

population-based, and conducted in farming areas. Potential confounding from multiple exposures was accounted for in the analysis.]

Using the data set of the pooled population-based case–control studies in Iowa, Minnesota, and Nebraska, USA, Lee *et al.* (2004a) investigated whether asthma acts as an effect modifier of the association between pesticide exposure and NHL. The study included 872 cases diagnosed with NHL from 1980 to 1986 and 2381 frequency-matched controls. Information on use of pesticides and history of asthma was based on interviews. A total of 177 subjects (45 cases, 132 controls) reported having been told by their doctor that they had asthma. Subjects with a history of asthma had a non-significantly lower risk of NHL than non-asthmatics, and there was no main effect of pesticide exposure. In general, asthmatics tended to have larger odds ratios associated with exposure to pesticides than non-asthmatics. There was no indication of effect modification: the odds ratio associated with glyphosate use was 1.4 (95% CI, 0.98–2.1; 53 exposed cases) among non-asthmatics and 1.2 (95% CI, 0.4–3.3; 6 exposed cases) for asthmatics, when compared with non-asthmatic non-exposed farmers). [This analysis overlapped with that of De Roos *et al.* (2003).]

(b) The cross-Canada case–control study

McDuffie *et al.* (2001) studied the associations between exposure to specific pesticides and NHL in a multicentre population-based study with 517 cases and 1506 controls among men of six Canadian provinces (see the *Monograph* on Malathion, Section 2.0, for a detailed description of this study). Odds ratios of 1.26 (95% CI, 0.87–1.80; 51 exposed cases; adjusted for age and province) and 1.20 (95% CI, 0.83–1.74, adjusted for age, province, high-risk exposures) were observed for exposure to glyphosate. In an analysis by frequency of exposure to glyphosate, participants with > 2 days of exposure per year had an odds ratio of 2.12 (95% CI, 1.20–3.73, 23 exposed cases) compared with those with some, but ≤ 2 days of exposure. [The study was large, but had relatively low participation rates.]

Kachuri *et al.* (2013) investigated the association between lifetime use of pesticides and multiple myeloma in a population-based case–control study among men in six Canadian provinces between 1991 and 1994 (see the *Monograph* on Malathion, Section 2.0, for a detailed description of this study). Data from 342 cases of multiple myeloma and 1357 controls were obtained for ever-use of pesticides, number of pesticides used, and days per year of pesticide use. The odds ratios were adjusted for age, province of residence, type of respondent, smoking and medical history. The odds ratio for ever-use of glyphosate was 1.19 (95% CI, 0.76–1.87; 32 cases). When the analysis was conducted by level of exposure, no association was found for light users (≤ 2 days per year) of glyphosate (OR, 0.72; 95% CI, 0.39–1.32; 15 exposed cases) while the odds ratio in heavier users (> 2 days per year) was 2.04 (95% CI, 0.98–4.23; 12 exposed cases). [The study had relatively low response rates. Multiple myeloma is now considered a subtype of NHL.]

(c) Case–control studies in Sweden

Nordström *et al.* (1998) conducted a population case–control study in Sweden on hairy cell leukaemia (considered to be a subgroup of NHL). The study included 121 cases in men and 484 controls matched for age and sex. An age-adjusted odds ratio of 3.1 (95% CI, 0.8–12; 4 exposed cases) was observed for exposure to glyphosate. [This study had limited power to detect an effect, and there was no adjustment for other exposures.]

Hardell & Eriksson (1999) reported the results of a population-based case–control study on the incidence of NHL in men associated with pesticide exposure in four northern counties in Sweden. Exposure data was collected by questionnaire (also supplemented by telephone interviews) from 404 cases (192 deceased) and 741

controls (matched by age, sex, county, and vital status). Increased risks of NHL were found for subjects exposed to herbicides and fungicides. The odds ratio for ever-use of glyphosate was 2.3 (95% CI, 0.4–13; 4 exposed cases) in a univariate analysis, and 5.8 (95% CI, 0.6–54) in a multivariable analysis. [The exposure frequency was low for glyphosate, and the study had limited power to detect an effect. The variables included in the multivariate analysis were not specified. This study may have overlapped partially with those of Hardell *et al.* (2002).]

Hardell *et al.* (2002) conducted a pooled analysis of two case–control studies, one on NHL (already reported in Hardell & Eriksson, 1999) and another on hairy cell leukaemia, a subtype of NHL (already reported by Nordström *et al.*, 1998). The pooled analysis of NHL and hairy cell leukaemia was based on 515 cases and 1141 controls. Increased risk was found for exposure to glyphosate (OR, 3.04; 95% CI, 1.08–8.52; 8 exposed cases) in the univariate analysis, but the odds ratio decreased to 1.85 (95% CI, 0.55–6.20) when study, study area, and vital status were considered in a multivariate analysis. [The exposure frequency was low for glyphosate and the study had limited power. This study partially overlapped with those of Hardell & Eriksson (1999) and Nordström *et al.* (1998).]

Eriksson *et al.* (2008) reported the results of a population based case–control study of exposure to pesticides as a risk factor for NHL. Men and women aged 18–74 years living in Sweden were included from 1 December 1999 to 30 April 2002. Incident cases of NHL were enrolled from university hospitals in Lund, Linköping, Örebro, and Umeå. Controls (matched by age and sex) were selected from the national population registry. Exposure to different agents was assessed by questionnaire. In total, 910 (91%) cases and 1016 (92%) controls participated. Multivariable models included agents with statistically significant increased odds ratios (MCPA, 2-methyl-4-chlorophenoxyacetic acid), or with an odds ratio of > 1.50 and at least 10 exposed subjects (2,4,5-T and/or 2,4-D; mercurial seed dressing, arsenic, creosote, tar), age, sex, year of diagnosis or enrolment. The odds ratio for exposure to glyphosate was 2.02 (95% CI, 1.10–3.71) in a univariate analysis, and 1.51 (95% CI, 0.77–2.94) in a multivariable analysis. When exposure for more than 10 days per year was considered, the odds ratio was 2.36 (95% CI, 1.04–5.37). With a latency period of > 10 years, the odds ratio was 2.26 (95% CI, 1.16–4.40). The associations with exposure to glyphosate were reported also for lymphoma subtypes, and elevated odds ratios were reported for most of the cancer forms, including B-cell lymphoma (OR, 1.87; 95% CI, 0.998–3.51) and the subcategory of small lymphocytic lymphoma/chronic lymphocytic leukaemia (OR, 3.35; 95% CI, 1.42–7.89; [not adjusted for other pesticides]). [This was a large study; there was possible confounding from use of other pesticides including MCPA, but this was considered in the analysis.]

(d) Other case–control studies in Europe

Orsi *et al.* (2009) reported the results of a hospital-based case–control study conducted in six centres in France between 2000 and 2004. Incident cases with a diagnosis of lymphoid neoplasm aged 20–75 years and controls of the same age and sex as the cases were recruited in the same hospital, mainly in the orthopaedic and rheumatological departments during the same period. [The Working Group noted that the age of case eligibility was given in the publication as 20–75 years in the materials and methods section, but as 18–75 years in the abstract.] Exposures to pesticides were evaluated through specific interviews and case-by-case expert reviews. The analyses included 491 cases (244 cases of NHL, 87 cases of Hodgkin lymphoma), 104 of lymphoproliferative syndrome, and 56 cases of multiple myeloma), and 456 age- and sex-matched controls. Positive associations between some subtypes and occupational exposure to several pesticides

were noted. The odds ratios associated with any exposure to glyphosate were 1.2 (95% CI, 0.6–2.1; 27 exposed cases) for all lymphoid neoplasms combined, 1.0 (95% CI, 0.5–2.2; 12 exposed cases) for NHL, 0.6 (95% CI, 0.2–2.1; 4 exposed cases) for lymphoproliferative syndrome, 2.4 (95% CI, 0.8–7.3) for multiple myeloma, and 1.7 (95% CI, 0.6–5.0; 6 exposed cases) for Hodgkin lymphoma, after adjusting for age, centre, and socioeconomic category ("blue/white collar").

Cocco *et al.* (2013) reported the results of a pooled analysis of case–control studies conducted in six European countries in 1998–2004 (EPILYMPH, Czech Republic, France, Germany, Ireland, Italy, and Spain) to investigate the role of occupational exposure to specific groups of chemicals in the etiology of lymphoma overall, B-cell lymphoma, and its most prevalent subtypes. A total of 2348 incident cases of lymphoma and 2462 controls were recruited. Controls from Germany and Italy were randomly selected by sampling from the general population, while the rest of the centres used matched hospital controls. Overall, the participation rate was 88% for cases, 81% for hospital controls, and 52% for population controls. An occupational history was collected with farm work-specific questions on type of crop, farm size, pests being treated, type and schedule of pesticide use. In each study centre, industrial hygienists and occupational experts assessed exposure to specific groups of pesticides and individual compounds with the aid of agronomists. [Therefore any exposure misclassification would be non-differential.] Analyses were conducted for lymphoma and the most prevalent lymphoma subtypes adjusting for age, sex, education, and centre. Lymphoma overall, and B-cell lymphoma were not associated with any class of the investigated pesticides, while the risk of chronic lymphocytic leukaemia was elevated among those ever exposed to inorganic and organic pesticides. Only for a few individual agrochemicals was there a sizeable number of study subjects to conduct a meaningful analysis, and the odds ratio for exposure to glyphosate and B-cell lymphoma was 3.1 (95% CI, 0.6–17.1; 4 exposed cases and 2 exposed controls). [The study had a very limited power to assess the effects of glyphosate on risk of NHL.]

2.2.2 Other haematopoietic cancers

Orsi *et al.* (2009) also reported results for Hodgkin lymphoma (see Section 2.2.1).

Karunanayake *et al.* (2012) conducted a case–control study of Hodgkin lymphoma among white men, aged 19 years or older, in six regions of Canada (see the Malathion *Monograph*, Section 2.0, for a detailed description of this study). The analysis included 316 cases and 1506 age-matched (± 2 years) controls. Based on 38 cases exposed to glyphosate, the odds ratios were 1.14 (95% CI, 0.74–1.76) adjusted for age and province, and 0.99 (95% CI, 0.62–1.56) when additionally adjusted for medical history variables.

Brown *et al.* (1990) evaluated exposure to carcinogens in an agricultural setting and the relationship with leukaemia in a population-based case–control interview study in Iowa and Minnesota, USA, including 578 white men with leukaemia and 1245 controls. The exposure assessment was done with a personal interview of the living subjects or the next-of-kin. Farmers had a higher risk of all leukaemias compared with non-farmers, and associations were found for exposure to specific animal insecticides, including the organophosphates crotoxyphos, dichlorvos, famphur, pyrethrins, and methoxychlor. The odds ratio for glyphosate was 0.9 (95% CI, 0.5–1.6; 15 exposed cases; adjusted for vital status, age, state, tobacco use, family history of lymphopoietic cancer, high-risk occupations, and high-risk exposures). [This was a large study in an agricultural setting, but had limited power for studying the effects of glyphosate use.]

2.3 Case–control studies on other cancer sites

2.3.1 Cancer of the oesophagus and stomach

Lee *et al.* (2004b) evaluated the risk of adenocarcinomas of the oesophagus and stomach associated with farming and agricultural pesticide use. The population-based case–control study was conducted in eastern Nebraska, USA. Subjects of both sexes diagnosed with adenocarcinoma of the stomach (n = 170) or oesophagus (n = 137) between 1988 and 1993 were enrolled. Controls (n = 502) were randomly selected from the population registry of the same geographical area. The response rates were 79% for cancer of the stomach, 88% for cancer of the oesophagus, and 83% for controls. Adjusted odds ratios were estimated for use of individual and chemical classes of insecticides and herbicides, with non-farmers as the reference category. No association was found with farming or ever-use of insecticides or herbicides, or with individual pesticides. For ever-use of glyphosate, the odds ratio was 0.8 (95% CI, 0.4–1.4; 12 exposed cases) for cancer of the stomach, and 0.7 (95% CI, 0.3–1.4; 12 exposed cases) for oesophageal cancer. [The study was conducted in a farming area, but the power to detect an effect of glyphosate use was limited.]

2.3.2 Cancer of the brain

Ruder *et al.* (2004) conducted a case–control study on glioma among nonmetropolitan residents of Iowa, Michigan, Minnesota, and Wisconsin in the Upper Midwest Health Study, USA. The study included 457 cases of glioma and 648 population-based controls, all adult men. Exposure assessment was done with interviews of the subject or the relatives. The response rates were 93% and 70% for cases and controls, respectively. No association were found with any of the pesticides assessed, including glyphosate. [Glyphosate use was assessed, but specific results were not presented.]

Carreón *et al.* (2005) evaluated the effects of rural exposures to pesticides on risk of glioma among women aged 18–80 years who were nonmetropolitan residents of Iowa, Michigan, Minnesota, and Wisconsin in the Upper Midwest Health Study, USA. A total of 341 cases of glioma and 528 controls were enrolled. A personal interview was carried out for exposure assessment. The response rates were 90% and 72%, respectively. After adjusting for age, age group, education, and farm residence, no association with glioma was observed for exposure to several pesticide classes or individual pesticides. There was a reduced risk for glyphosate (OR, 0.7; 95% CI, 0.4–1.3; 18 exposed cases). These results were not affected by the exclusion of proxy respondents (43% of cases, 2% of controls).

Lee *et al.* (2005) evaluated the association between farming and agricultural pesticide use and risk of adult glioma in a population-based case–control study in eastern Nebraska, USA. Cases of glioma were in men and women (n = 251) and were compared with population controls from a previous study (n = 498). A telephone interview was conducted for 89% of the cases and 83% of the controls. Adjusted odds ratios for farming and for use of individual and chemical classes of insecticides and herbicides were calculated using non-farmers as the reference category. Among men, ever living or working on a farm and duration of farming were associated with significantly increased risks of glioma, but the positive findings were limited to proxy respondents. Among women, there were no positive associations with farming activities among self or proxy respondents. Some specific pesticide families and individual pesticides were associated with significantly increased risks among male farmers, but most of the positive associations were limited to proxy respondents. There was a non-significant excess risk with glyphosate use for the overall group (OR, 1.5; 95% CI, 0.7–3.1; 17 exposed cases), but there was inconsistency between observations for self-respondents (OR,

0.4; 95% CI, 0.1–1.6) and observations for proxy respondents (OR, 3.1; 95% CI, 1.2–8.2). [The study had limited power to detect an effect of glyphosate use, and the inconsistencies for self and proxy respondents made the results difficult to interpret.]

2.3.3 Soft tissue sarcoma

Pahwa *et al.* (2011) reported the results of the soft tissue sarcoma component of the cross-Canada study in relation to specific pesticides, including 357 cases of soft tissue sarcoma and 1506 population controls from 1991–1994. The fully adjusted odds ratio for glyphosate use was 0.90 (95% CI, 0.58–1.40).

2.3.4 Cancer of the prostate

Band *et al.* (2011) report results of a case–control study including 1516 patients with cancer of the prostate (ascertained by the cancer registry of British Columbia, Canada, for 1983–90) and 4994 age-matched controls with cancers at all other cancer sites excluding lung and unknown primary site. Agricultural exposures were assessed by job-exposure matrix. A total of 60 cases were exposed to glyphosate (adjusted OR, 1.36; 95% CI, 0.83–2.25).

2.3.5 Childhood cancer

Parental exposure to pesticides, including glyphosate, was assessed in a population-based case–control study of childhood leukaemia in Costa Rica (Monge *et al.*, 2007). However, associations of childhood cancer with glyphosate were reported only for an "other pesticides" category that also included paraquat, chlorothalonil, and other chemicals. [Because glyphosate was not specifically assessed, this study was not evaluated by the Working Group.]

2.4. Meta-analyses

Schinasi & Leon (2014) conducted a systematic review and meta-analysis of NHL and occupational exposure to agricultural pesticides, including glyphosate. The meta-analysis for glyphosate included six studies (McDuffie *et al.*, 2001; Hardell *et al.*, 2002; De Roos *et al.*, 2003; 2005a; Eriksson *et al.*, 2008; Orsi *et al.*, 2009) and yielded a meta risk-ratio of 1.5 (95% CI, 1.1–2.0). [The Working Group noted that the most fully adjusted risk estimates from the articles by Hardell *et al.* (2002) and Eriksson *et al.* (2008) were not used in this analysis. After considering the adjusted estimates of the two Swedish studies in the meta-analysis, the Working Group estimated a meta risk-ratio of 1.3 (95% CI, 1.03–1.65), I^2 = 0%, *P* for heterogeneity 0.589.]

3. Cancer in Experimental Animals

3.1 Mouse

See Table 3.1

3.1.1 Dietary administration

Groups of 50 male and 50 female CD-1 mice [age not reported] were given diets containing glyphosate (purity, 99.7%) at a concentration of 0, 1000, 5000, or 30 000 ppm, ad libitum, for 24 months. There was no treatment-related effect on body weight in male and female mice at the lowest or intermediate dose. There was a consistent decrease in body weight in the male and female mice at the highest dose compared with controls. Survival in all dose groups was similar to that of controls. There was a positive trend (P = 0.016, trend test; see EPA, 1985b) in the incidence of renal tubule adenoma in dosed male mice: 0/49, 0/49, 1/50 (2%), 3/50 (6%). [The Working Group noted that renal tubule adenoma is a rare tumour in CD-1 mice.] No data on tumours of the kidney

Table 3.1 Studies of carcinogenicity with glyphosate in mice

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	For each target organ: incidence (%) and/or multiplicity of tumours	Significance	Comments
Mouse, CD-1 (M, F) 24 mo EPA (1985a, b, 1986, 1991a)	Diet containing glyphosate (technical grade; purity, 99.7%) at concentrations of 0, 1000, 5000, or 30 000 ppm, ad libitum, for 24 mo 50 M and 50 F/group [age, NR]	*Males* Renal tubule adenoma: 0/49, 0/49, 1/50 (2%), 3/50 (6%) *Females* No data provided on the kidney Report from the PWG of the EPA (1986): *Males* Renal tubule adenoma: 1/49 (2%), 0/49, 0/50, 1/50 (2%) Renal tubule carcinoma: 0/49, 0/49, 1/50 (2%), 2/50 (4%) Renal tubule adenoma or carcinoma (combined): 1/49 (2%), 0/49, 1/50 (2%), 3/50 (6%)	*P* for trend = 0.016; see Comments [NS] [*P* = 0.037; Cochran–Armitage trend test] [*P* = 0.034; Cochran–Armitage trend test]	No information was provided on renal tubule adenomas in female mice, or on statistical analyses of tumour data EPA recommended that additional renal sections be cut and evaluated from all control and treated male mice. The pathology report for these additional sections (EPA, 1985b) showed the same incidence of renal tubule adenomas as originally reported, with no significant difference in incidence when comparing control and treated groups; however, the test for linear trend in proportions resulted in *P* = 0.016 EPA (1986) convened a PWG and requested additional pathological and statistical information on kidney tumours observed in male mice treated with glyphosate
Mouse, CD-1 (M, F) 104 wk JMPR (2006)	Diet containing glyphosate (purity, 98.6%) at doses of 0, 100, 300, 1000 mg/kg bw, ad libitum, for 104 wk 50 M and 50 F/group [age, NR]	*Males* Haemangiosarcoma: 0/50, 0/50, 0/50, 4/50 (8%) Histiocytic sarcoma in the lymphoreticular/haemopoietic tissue: 0/50, 2/50 (4%), 0/50, 2/50 (4%) *Females* Haemangiosarcoma: 0/50, 2/50 (4%), 0/50, 1/50 (2%) Histiocytic sarcoma in the lymphoreticular/haemopoietic tissue: 0/50, 3/50 (6%), 3/50 (6%), 1/50 (2%)	[*P* < 0.001; Cochran–Armitage trend test] NS NS NS	

Case 3:16-md-02741-VC Document 654-2 Filed 10/28/17 Page 77 of 138

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	For each target organ: incidence (%) and/or multiplicity of tumours	Significance	Comments
Mouse, Swiss (M) 32 wk George *et al.* (2010)	Initiation–promotion study Skin application of glyphosate-based formulation (glyphosate, 41%; POEA, ~15%) (referred to as "glyphosate") dissolved in 50% ethanol; DMBA dissolved in 50% ethanol, and TPA dissolved in 50% acetone, used in the groups described below 20 M/group	Skin tumours [called "papillomas" by the authors, following gross examination only]		Short duration of treatment, no solvent controls, and lack of any histopathological evaluation Age at start, NR (mice weighed 12–15 g bw) [The Working Group concluded this was an inadequate study for the evaluation of glyphosate]
	Group I: untreated control (no treatment)	Group I: 0/20		
	Group II: glyphosate only: 25 mg/kg bw topically, 3 × /wk, for 32 wk	Group II: 0/20		
	Group III: single topical application of DMBA, 52 µg/mouse, followed 1 wk later by TPA, 5 µg/mouse, 3 × /wk, for 32 wk	Group III: 20/20*, 7.8 ± 1.1	*$P < 0.05$ vs groups VI and VII	
	Group IV: single topical application of glyphosate, 25 mg/kg bw, followed 1 wk later by TPA, 5 µg/mouse, 3 × /wk, for 32 wk	Group I: 0/20		
	Group V: 3 × /wk topical application of glyphosate, 25 mg/kg bw, for 3 wk, followed 1 wk later by TPA, 5 µg/mouse, 3 × /wk, for 32 wk	Group V: 0/20		
	Group VI: single topical application of DMBA, 52 µg/mouse	Group VI: 0/20		
	Group VII: topical application of TPA, 5 µg/mouse, 3 × /wk, for 32 wk	Group VII: 0/20		
	Group VIII: single topical application of DMBA, 52 µg/mouse, followed 1 wk later by topical treatment with glyphosate, 25 mg/kg bw, 3 × /wk, for 32 wk	Group VIII: 8/20*, 2.8 ± 0.9	*$P < 0.05$ vs group VI	

bw, body weight; DMBA, 7,12-dimethylbenz[*a*]anthracene; EPA, United States Environmental Protection Agency; F, female; M, male; mo, month; NR, not reported; NS, not significant; POEA, polyethoxylated tallowamine; PWG, pathology working group; TPA, 12-*O*-tetradecanoyl-phorbol-13-acetate; vs, versus; wk, week; yr, year

Case 3:16-md-02741-VC Document 654-2 Filed 10/28/17 Page 78 of 138

were provided for female mice. No other tumour sites were identified (EPA, 1985a). Subsequent to its initial report (EPA, 1985a), the United States Environmental Protection Agency (EPA) recommended that additional renal sections be cut and evaluated from all male mice in the control and treated groups. The pathology report for these additional sections (EPA, 1985b) indicated the same incidence of renal tubule adenoma as originally reported, with no significant increase in incidence between the control group and treated groups by pairwise comparison. However, as already reported above, the test for linear trend in proportions resulted in a significance of $P = 0.016$. The EPA (1986) also requested that a pathology working group (PWG) be convened to evaluate the tumours of the kidney observed in male mice treated with glyphosate, including the additional renal sections. In this second evaluation, the PWG reported that the incidence of adenoma of the renal tubule was 1/49 (2%), 0/49, 0/50, 1/50 (2%) [not statistically significant]; the incidence of carcinoma of the renal tubule was 0/49, 0/49, 1/50 (2%), 2/50 (4%) [$P = 0.037$, trend test for carcinoma]; and the incidence of adenoma or carcinoma (combined) of the renal tubule was 1/49 (2%), 0/49, 1/50 (2%), 3/50 (6%) [$P = 0.034$, trend test for combined]. [The Working Group considered that this second evaluation indicated a significant increase in the incidence of rare tumours, with a dose-related trend, which could be attributed to glyphosate. Chandra & Frith (1994) reported that only 1 out of 725 [0.14%] CD-1 male mice in their historical database had developed renal cell tumours (one carcinoma).]

[The Working Group noted the differences in histopathological diagnosis between pathologists. Proliferative lesions of the renal tubules are typically categorized according to published criteria as hyperplasia, adenoma, or carcinoma. The difference is not trivial, because focal hyperplasia, a potentially preneoplastic lesion, should be carefully differentiated from the regenerative changes of the tubular epithelium. There is a morphological continuum in the development and progression of renal neoplasia. Thus larger masses may exhibit greater heterogeneity in histological growth pattern, and cytologically more pleomorphism and atypia than smaller lesions (Eustis *et al.*, 1994). Of note, a renal tumour confirmed by the PWG after re-evaluation of the original slides (EPA, 1986), had not been seen in the re-sectioned kidney slides (EPA, 1985b). This may be related to the growth of tumour that – in contrast to tumours in other organs – is not spherical but elliptical because of the potential expansion in tubules. In addition, the concept of tubular expansion without compression of adjacent parenchyma may be at the basis of the discrepancy between the first (EPA, 1985a, b) and second evaluation (EPA, 1986).]

In another study reported to the Joint FAO/WHO Meeting on Pesticide Residues (JMPR), groups of 50 male and 50 female CD-1 mice [age at start not reported] were given diets containing glyphosate (purity, 98.6%) at a concentration that was adjusted weekly for the first 13 weeks and every 4 weeks thereafter to give doses of 0, 100, 300, or 1000 mg/kg bw, ad libitum, for 104 weeks (JMPR, 2006). There was no treatment-related effect on body weight or survival in any of the dosed groups. There was an increase in the incidence of haemangiosarcoma in males – 0/50, 0/50, 0/50, 4/50 (8%) [$P < 0.001$, Cochran–Armitage trend test], and in females – 0/50, 2/50 (4%), 0/50, 1/50 (2%) [not statistically significant], and an increase in the incidence of histiocytic sarcoma in the lymphoreticular/haemopoietic tissue in males – 0/50, 2/50 (4%), 0/50, 2/50 (4%), and in females – 0/50, 3/50 (6%), 3/50 (6%), 1/50 (2%) [not statistically significant for males or females]. [The Working Group considered that this study was adequately reported.]

3.1.2 Initiation–promotion

Groups of 20 male Swiss mice [age at start not reported; body weight, 12–15 g] were given a glyphosate-based formulation (glyphosate, 41%; polyethoxylated tallowamine, ~15%) (referred to as glyphosate in the article) that was dissolved in 50% ethanol and applied onto the shaved back skin (George *et al.*, 2010). Treatment groups were identified as follows:

- Group I – untreated control;
- Group II – glyphosate only (25 mg/kg bw), applied topically three times per week for 32 weeks;
- Group III – single topical application of dimethylbenz[*a*]anthracene (DMBA; in ethanol; 52 µg/mouse), followed 1 week later by 12-*O*-tetradecanoylphorbol-13-acetate (TPA; in acetone; 5 µg/mouse), applied topically three times per week for 32 weeks;
- Group IV – single topical application of glyphosate (25 mg/kg bw) followed 1 week later by TPA (in acetone; 5 µg/mouse), applied topically three times per week for 32 weeks;
- Group V – glyphosate (25 mg/kg bw) applied topically three times per week for 3 weeks (total of nine applications), followed 1 week later by TPA (in acetone; 5 µg/mouse), applied topically three times per week for 32 weeks;
- Group VI – single topical application of DMBA (in ethanol; 52 µg/mouse);
- Group VII –TPA (in acetone; 5 µg/mouse), applied topically three times per week for 32 weeks; and
- Group VIII –single topical application of DMBA (in ethanol; 52 µg/mouse), followed 1 week later by glyphosate (25 mg/kg bw), applied topically three times per week for 32 weeks.

All mice were killed at 32 weeks. Skin tumours were observed only in group III (positive control, DMBA + TPA, 20/20) and group VIII (DMBA + glyphosate, 8/20; $P < 0.05$ versus group VI [DMBA only, 0/20]). No microscopic examination was conducted and tumours were observed "as a minute wart like growth [that the authors called squamous cell papillomas], which progressed during the course of experiment." [The glyphosate formulation tested appeared to be a tumour promoter in this study. The design of the study was poor, with short duration of treatment, no solvent controls, small number of animals, and lack of histopathological examination. The Working Group concluded that this was an inadequate study for the evaluation of glyphosate.]

3.1.3 Review articles

Greim *et al.* (2015) have published a review article containing information on five long-term bioassay feeding studies in mice. Of these studies, one had been submitted for review to the EPA (EPA, 1985a, b, 1986, 1991a), and one to the JMPR (JMPR, 2006); these studies are discussed in Section 3.1.1. The review article reported on an additional three long-term bioassay studies in mice that had not been previously available in the open literature, but had been submitted to various organizations for registration purposes. The review article provided a brief summary of each study and referred to an online data supplement containing the original data on tumour incidence from study reports. The three additional long-term bioassay studies in mice are summarized below. [The Working Group was unable to evaluate these studies, which are not included in Table 3.1 and Section 5.3, because the information provided in the review article and its supplement was insufficient (e.g. information was lacking on statistical methods, choice of doses, body-weight gain, survival data, details of histopathological examination, and/or stability of dosed feed mixture).]

In the first study (identified as Study 12, 1997a), groups of 50 male and 50 female CD-1

mice [age at start not reported] were given diets containing glyphosate (purity, 94–96%) at a concentration of 0, 1600, 8000, or 40 000 ppm for 18 months. The increase in the incidence of bronchiolo-alveolar adenoma and carcinoma, and of lymphoma, was reported to be not statistically significant in males and females receiving glyphosate. [The Working Group was unable to evaluate this study because of the limited experimental data provided in the review article and supplemental information.]

In the second study (identified as Study 13, 2001), groups of 50 male and 50 female Swiss albino mice [age at start not reported] were given diets containing glyphosate (purity, > 95%) at a concentration of 0 (control), 100, 1000, or 10 000 ppm for 18 months. The authors reported a statistically significant increase in the incidence of malignant lymphoma (not otherwise specified, NOS) in males at the highest dose: 10/50 (20%), 15/50 (30%), 16/50 (32%), 19/50 (38%; $P < 0.05$; pairwise test); and in females at the highest dose: 18/50 (36%), 20/50 (40%), 19/50 (38%), 25/50 (50%; $P < 0.05$; pairwise test). [The Working Group was unable to evaluate this study because of the limited experimental data provided in the review article and supplemental information.]

In the third study (identified as Study 14, 2009a), groups of 51 male and 51 female CD-1 mice [age at start not reported] were given diets containing glyphosate (purity, 94.6–97.6%) at a concentration of 0, 500, 1500, or 5000 ppm for 18 months. Incidences for bronchiolo-alveolar adenoma and carcinoma, malignant lymphoma (NOS), and hepatocellular adenoma and carcinoma in males, and for bronchiolo-alveolar adenoma and carcinoma, malignant lymphoma (NOS) and pituitary adenoma in females, were included in the article. In males, the authors reported that there was a significant positive trend [statistical test not specified] in the incidence of bronchiolo-alveolar carcinoma (5/51, 5/51, 7/51, 11/51) and of malignant lymphoma (0/51, 1/51, 2/51, 5/51). [The Working Group was unable to evaluate this study because of the limited experimental data provided in the review article and supplemental information.]

3.2 Rat

See Table 3.2

3.2.1 Drinking-water

Groups of 10 male and 10 female Sprague-Dawley rats (age, 5 weeks) were given drinking-water containing a glyphosate-based formulation at a dose of 0 (control), 1.1×10^{-8}% (5.0×10^{-5} mg/L), 0.09% (400 mg/L) or 0.5% (2.25×10^{3} mg/L), ad libitum, for 24 months (Séralini *et al.*, 2014). [The study reported is a life-long toxicology study on a glyphosate-based formulation and on genetically modified NK603 maize, which the authors stated was designed as a full study of long-term toxicity and not a study of carcinogenicity. No information was provided on the identity or concentration of other chemicals contained in this formulation.] Survival was similar in treated and control rats. [No data on body weight were provided.] In female rats, there was an almost twofold increase in the incidence of tumours of the mammary gland (mainly fibroadenoma and adenocarcinoma) in animals exposed to the glyphosate-based formulation only versus control animals: control, 5/10 (50%); lowest dose, 9/10 (90%); intermediate dose, 10/10 (100%) [$P < 0.05$; Fisher exact test]; highest dose, 9/10 (90%). [The Working Group concluded that this study conducted on a glyphosate-based formulation was inadequate for evaluation because the number of animals per group was small, the histopathological description of tumours was poor, and incidences of tumours for individual animals were not provided.]

In another study with drinking-water, Chruscielska *et al.* (2000) gave groups of 55 male and 55 female Wistar rats (age, 6–7 weeks) drinking-water containing an ammonium salt

of glyphosate as a 13.85% solution [purity of glyphosate, not reported] that was used to make aqueous solutions of 0 (control), 300, 900, and 2700 mg/L, for 24 months [details on the dosing regimen were not reported]. The authors reported that survival and body-weight gain were similar in treated and control animals. No significant increase in tumour incidence was reported in any of the treated groups. [The Working Group noted the limited information provided on dosing regimen, histopathological examination method, and tumour incidences.]

3.2.2 Dietary administration

The JMPR report included information on a 1-year feeding study in which groups of 24 male and 24 female Wistar-Alpk:APfSD rats [age at start not reported] were given diets containing glyphosate (purity, 95.6%) at a concentration of 0, 2000, 8000, or 20 000 ppm, ad libitum, for 1 year (JMPR, 2006). There was a treatment-related decrease in body-weight gain at the two highest doses (significant at 20 000 ppm for both sexes, and at 8000 ppm only in females). There was no treatment-related decrease in survival. No significant increase in tumour incidence was observed in any of the treated groups. [The Working Group noted the short duration of exposure.]

The JMPR report also included information on a 104-week feeding study in which groups of 50 male and 50 female Sprague-Dawley rats [age at start not reported] were given diets containing glyphosate (purity, 98.7–98.9%) at a concentration that was adjusted to provide doses of 0, 10, 100, 300, or 1000 mg/kg bw, ad libitum, for 104 weeks (JMPR, 2006). There was a treatment-related decrease in body-weight gain in males and females at the highest dose. There was no significant treatment-related decrease in survival or increase in tumour incidence in any of the treated groups.

Information was also included in the JMPR report on a 24-month feeding study in which groups of 52 male and 52 female Wistar-Alpk:APfSD rats [age at start not reported] were given diets containing glyphosate (purity, 97.6%) at a concentration of 0, 2000, 6000, or 20 000 ppm, ad libitum, for 24 months (JMPR, 2006). There was a treatment-related decrease in body-weight gain in males and females at the highest dose, and a corresponding significant increase in survival in males. No significant increase in tumour incidence was observed in any of the treated groups.

The EPA (1991a, b, c, d) provided information on a long-term study in which groups of 60 male and 60 female Sprague-Dawley rats (age, 8 weeks) were given diets containing glyphosate (technical grade; purity, 96.5%) at a concentration of 0 ppm, 2000 ppm, 8000 ppm, or 20 000 ppm, ad libitum, for 24 months. Ten animals per group were killed after 12 months. There was no compound-related effect on survival, and no statistically significant decreases in body-weight gain in male rats. In females at the highest dose, body-weight gain was significantly decreased, starting on day 51. In males at the lowest dose, there was a statistically significant increase in the incidence of pancreatic islet cell adenoma compared with controls: 8/57 (14%) versus 1/58 (2%), $P \leq 0.05$ (Fisher exact test). Additional analyses by the EPA (1991a) (using the Cochran–Armitage trend test and Fisher exact test, and excluding rats that died or were killed before week 55) revealed a statistically significant higher incidence of pancreatic islet cell adenoma in males at the lowest and highest doses compared with controls: lowest dose, 8/45 (18%; $P = 0.018$; pairwise test); intermediate dose, 5/49 (10%); highest dose, 7/48 (15%; $P = 0.042$; pairwise test) versus controls, 1/43 (2%). The range for historical controls for pancreatic islet cell adenoma reported in males at this laboratory was 1.8–8.5%. [The Working Group noted that there was no statistically significant positive trend in the incidence of these tumours, and no apparent progression to carcinoma.] There was also a statistically significant positive trend in the incidence of hepatocellular adenoma in

Table 3.2 Studies of carcinogenicity with glyphosate in rats

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	For each target organ: incidence (%) and/or multiplicity of tumours	Significance	Comments
Rat, Sprague-Dawley (M, F) 24 mo Séralini *et al.* (2014)	Drinking-water containing a glyphosate-based formulation at a concentration of 0 (control), 1.1×10^{-8}% (glyphosate, 5.0×10^{-5} mg/L), 0.09% (glyphosate, 400 mg/L) or 0.5% (glyphosate, 2.25×10^{3} mg/L), ad libitum, for 24 mo 10 M and 10 F/group (age, 5 wk)	*Males* No significant increase in tumour incidence observed in any of the treated groups *Females* Mammary tumours (mainly fibroadenomas and adenocarcinomas): 5/10 (50%), 9/10 (90%), 10/10 (100%)*, 9/10 (90%) Pituitary lesions (hypertrophy, hyperplasia, and adenoma): 6/10 (60%), 8/10 (80%), 7/10 (70%), 7/10 (70%)	NS *[$P < 0.05$] [NS]	Data are from an in-depth life-long toxicology study on a glyphosate-based formulation and NK603 genetically modified maize; authors stated that the study was designed as a full chronic toxicity and not a carcinogenicity study. No information provided on the identity or concentration of other chemicals contained in this formulation Histopathology poorly described and tumour incidences for individual animals not discussed in detail. Small number of animals per group [The Working Group concluded this was an inadequate study for the evaluation of glyphosate carcinogenicity]
Rat, Wistar (M, F) 24 mo Chruscielska *et al.* (2000)	Drinking-water containing ammonium salt of glyphosate (13.85% solution) [purity of glyphosate, NR] was used to make aqueous solutions of 0, 300, 900, and 2700 mg/L [Details on dosing regimen, NR] 55 M and 55 F/group (age, 6–7 wk)	No significant increase in tumour incidence observed in any of the treated groups	NS	Limited information on dosing regimen, histopathological examination methods, and tumour incidences
Rat, Wistar-Alpk:APfSD (M, F) 1 yr JMPR (2006)	Diet containing glyphosate (purity, 95.6%) at concentrations of 0, 2000, 8000, or 20 000 ppm, ad libitum, for 1 yr 24 M and 24 F/group [age, NR]	No significant increase in tumour incidence observed in any groups of treated animals	NS	Short duration of exposure
Rat, Sprague-Dawley (M, F) 104 wk JMPR (2006)	Diet containing glyphosate (purity, 98.7–98.9%) at doses of 0, 10, 100, 300, or 1000 mg/kg bw, ad libitum, for 104 wk 50 M and 50 F/group [age, NR]	No significant increase in tumour incidence observed in any groups of treated animals	NS	
Rat, Wistar-Alpk:APfSD (M, F) 24 mo JMPR (2006)	Diet containing glyphosate (purity, 97.6%) at concentrations of 0, 2000, 6000, or 20 000 ppm, ad libitum, for 2 yr 52 M and 52 F/group [age, NR]	No significant increase in tumour incidence observed in any groups of treated animals	NS	

Case 3:16-md-02741-VC Document 654-2 Filed 10/28/17 Page 83 of 138

Case 3:16-md-02741-VC Document 654-2 Filed 10/28/17 Page 84 of 138

Table 3.2 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	For each target organ: incidence (%) and/or multiplicity of tumours	Significance	Comments
Rat Sprague-Dawley (M, F) 24 mo EPA (1991a, b, c, d)	Diet containing glyphosate (technical grade; purity, 96.5%) at concentrations of 0, 2000, 8000, or 20 000 ppm, ad libitum, for 24 mo 60 M and 60 F/group (age, 8 wk) 10 rats/group killed after 12 mo	*Males* *Pancreas (islet cell):* Adenoma: 1/58 (2%), 8/57 (14%)*, 5/60 (8%), 7/59 (12%) Carcinoma: 1/58 (2%), 0/57, 0/60, 0/59 Adenoma or carcinoma (combined): 2/58 (3%), 8/57 (14%), 5/60 (8%), 7/59 (12%) *Liver:* Hepatocellular adenoma: 2/60 (3%), 2/60 (3%), 3/60 (6%), 7/60 (12%) Hepatocellular carcinoma: 3/60 (5%), 2/60 (3%), 1/60 (2%), 2/60 (3%) *Females* *Pancreas (islet cell):* Adenoma: 5/60 (8%), 1/60 (2%), 4/60 (7%), 0/59 Carcinoma: 0/60, 0/60, 0/60, 0/59 Adenoma or carcinoma (combined): 5/60 (8%), 1/60 (2%), 4/60 (7%), 0/59 *Thyroid:* C-cell adenoma: 2/60 (3%), 2/60 (3%), 6/60 (10%), 6/60 (10%) C-cell carcinoma: 0/60, 0/60, 1/60, 0/60	Adenoma, * $P \leq 0.05$ (Fisher exact test with Bonferroni inequality); see comments Adenoma, *P* for trend = 0.016; see comments NS Adenoma, *P* for trend = 0.031; see comments	Historical control range for pancreatic islet cell adenoma reported in males at this laboratory, 1.8–8.5% EPA (1991a) performed additional analyses using the Cochran–Armitage trend test and Fisher exact test, and excluding animals that died or were killed before wk 54–55: *Males* *Pancreas (islet cell):* Adenoma: 1/43 (2%), 8/45 (18%; *P* = 0.018), 5/49 (10%), 7/48 (15%; *P* = 0.042) Carcinoma: 1/43 (2%), 0/45 (0%), 0/49 (0%), 0/48 (0%) Adenoma or carcinoma (combined): 2/43 (5%), 8/45 (18%), 5/49 (10%), 7/48 (15%) [There was no statistically significant positive trend in the incidence of pancreatic tumours, and no apparent progression to carcinoma] *Liver:* Hepatocellular adenoma: 2/44 (5%; *P* for trend = 0.016), 2/45 (4%), 3/49 (6%), 7/48 (15%) Hepatocellular carcinoma: 3/44 (7%); 2/45 (4%), 1/49 (2%), 2/48 (4%) Hepatocellular adenoma or carcinoma (combined): 5/44 (11%), 4/45 (9%), 4/49 (8%), 9/48 (19%) [There was no apparent progression to carcinoma] *Females* *Thyroid:* C-cell adenoma: 2/57 (4%; *P* for trend = 0.031), 2/60 (3%), 6/59 (10%), 6/55 (11%) C-cell carcinoma: 0/57, 0/60, 1/59 (2%), 0/55 C-cell adenoma or carcinoma (combined): 2/57 (4%), 2/60 (3%), 7/59 (12%), 6/55 (11%) [There was no apparent progression to carcinoma]

Table 3.2 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	For each target organ: incidence (%) and/or multiplicity of tumours	Significance	Comments
Rat Sprague-Dawley (M, F) Lifetime (up to 26 mo) EPA (1991a, b, c, d)	Diet containing glyphosate (purity, 98.7%) at concentrations of 0 ppm, 30 ppm (3 mg/kg bw per day), 100 ppm (10 mg/kg bw per day), 300 ppm (31 mg/kg bw per day), ad libitum, up to 26 mo 50 M and 50 F/group [age, NR]	*Males* *Pancreas (islet cell):* Adenoma: 0/50 (0%), 5/49* (10%), 2/50 (4%), 2/50 (4%) Carcinoma: 0/50 (0%), 0/49 (0%), 0/50 (0%), 1/50 (2%) Adenoma or carcinoma (combined): 0/50 (0%), 5/49 (10%), 2/50 (4%), 3/50 (6%) *Females* *Pancreas (islet cell):* Adenoma: 2/50 (4%), 1/50 (2%), 1/50 (2%), 0/50 (0%) Carcinoma: 0/50 (0%), 1/50 (2%), 1/50 (2%), 1/50 (2%) Adenoma or carcinoma (combined): 2/50 (10%), 2/50 (2%), 2/50 (74%), 1/50 (2%)	Adenoma, *[$P < 0.05$; Fisher exact test] NS	[There was no statistically significant positive trend in the incidence of pancreatic tumours, and no apparent progression to carcinoma]

bw, body weight; d, day; F, female; M, male; mo, month; NR, not reported; NS, not significant; wk, week; yr, year

Case 3:16-md-02741-VC Document 654-2 Filed 10/28/17 Page 85 of 138

males (P = 0.016) and of thyroid follicular cell adenoma in females (P = 0.031). [The Working Group noted that there was no apparent progression to carcinoma for either tumour type.]

The EPA (1991a, b, c, d) provided information on another long-term study in which groups of 50 male and 50 female Sprague-Dawley rats [age at start not reported] were given diets containing glyphosate (purity, 98.7%) at a concentration of 0, 30 (3 mg/kg bw per day), 100 (10 mg/kg bw per day), or 300 ppm (31 mg/kg bw per day), ad libitum, for life (up to 26 months). No information was provided on body weight or survival of the study animals. An increase in the incidence of pancreatic islet cell adenoma was reported in males at the lowest dose: controls, 0/50 (0%); lowest dose, 5/49 (10%) [$P < 0.05$; Fisher exact test]; intermediate dose, 2/50 (4%); highest dose, 2/50 (4%). [The Working Group noted that there was no statistically significant positive dose-related trend in the incidence of these tumours, and no apparent progression to carcinoma.]

3.2.3 Review articles

Greim *et al.* (2015) have published a review article containing information on nine long-term bioassay feeding studies in rats. Of these studies, two had been submitted for review to the EPA (1991a, b, c, d), two to the JMPR (JMPR, 2006), and one had been published in the openly available scientific literature (Chruscielska *et al.*, 2000); these studies are discussed earlier in Section 3.2. The review article reported on an additional four long-term bioassay studies in rats that had not been previously published, but had been submitted to various organizations for registration purposes. The review article provided a brief summary of each study and referred to an online data supplement containing the original data on tumour incidence from study reports. The four additional long-term bioassay studies in rats are summarized below. [The Working Group did not evaluate these studies, which are not included in Table 3.2 and Section 5.3, because the information provided in the review article and its supplement was insufficient (e.g. information lacking on statistical methods, choice of doses, body-weight gain, survival data, details on histopathological examination and/or stability of dosed feed mixture).]

In one study (identified as Study 4, 1996), groups of 50 male and 50 female Wistar rats [age at start not reported] were given diets containing glyphosate (purity, 96%) at a concentration of 0, 100, 1000, or 10 000 ppm, ad libitum, for 24 months. It was reported that hepatocellular adenomas and hepatocellular carcinomas were found at non-statistically significant incidences in both males and females. There was no significant increase in tumour incidence in the treated groups. [The Working Group was unable to evaluate this study because of the limited experimental data provided in the review article and supplemental information.]

In one study in Sprague-Dawley rats (identified as Study 5, 1997), groups of 50 male and 50 female rats [age at start not reported] were given diets containing glyphosate technical acid [purity not reported] at a concentration of 0, 3000, 15 000, or 25 000 ppm, ad libitum, for 24 months. There was no significant increase in tumour incidence in the treated groups. [The Working Group was unable to evaluate this study because of the limited experimental data provided in the review article and supplemental information.]

In a second study in Sprague Dawley rats (identified as Study 6, 1997b), groups of 50 males and 50 females [age at start not reported] were given diets containing glyphosate (purity, 94.6–97.6%) at a concentration of 0, 3000, 10 000, or 30 000 ppm, ad libitum, for 24 months. Non-significant increases in tumour incidences compared with controls were noted for skin keratoacanthoma in males at the highest dose, and for fibroadenoma of the mammary gland in females at the lowest and intermediate doses. [The Working Group was unable to evaluate this

study because of the limited experimental data provided in the review article and supplemental information.]

In another study in male and female Wistar rats (identified as Study 8, 2009b), groups of 51 male and 51 female rats [age at start not reported] were fed diets containing glyphosate (purity, 95.7%) at a concentration of 0, 1500, 5000, or 15 000 ppm, ad libitum, for 24 months. The highest dose was progressively increased to reach 24 000 ppm by week 40. A non-significant increase in tumour incidence was noted for adenocarcinoma of the mammary gland in females at the highest dose (6/51) compared with controls (2/51). [The Working Group was unable to evaluate this study because of the limited experimental data provided in the review article and supplemental information. The Working Group noted that tumours of the mammary gland had been observed in other studies in rats reviewed for the present *Monograph*.]

4. Mechanistic and Other Relevant Data

4.1 Toxicokinetic data

4.1.1 Introduction

The herbicidal activity of glyphosate is attributed to interference with the production of essential aromatic amino acids (EPA, 1993b). In plants, glyphosate competitively inhibits the activity of enolpyruvylshikimate phosphate synthase, an enzyme that is not present in mammalian cells. Glyphosate is degraded by soil microbes to aminomethylphosphonic acid (AMPA) (see Fig. 4.1), a metabolite that can accumulate in the environment. In mammals, glyphosate is not metabolized efficiently, and is mainly excreted unchanged into the urine; however, it has been suggested that glyphosate can undergo gut microbial metabolism in humans (Motojyuku *et al.*, 2008) and rodents (Brewster *et al.*, 1991).

4.1.2 Absorption

(a) Humans

Data on the absorption of glyphosate via intake of food and water in humans were not available to the Working Group. Inhalation of glyphosate is considered to be a minor route of exposure in humans, because glyphosate is usually formulated as an isopropylamine salt with a very low vapour pressure (Tomlin, 2000).

In the Farm Family Exposure Study, 60% of farmers had detectable levels of glyphosate in 24-hour composite urine samples taken on the day they had applied a glyphosate-based formulation (Acquavella *et al.*, 2004). Farmers who did not use rubber gloves had higher urinary concentrations of glyphosate than those who did use gloves [indicating that dermal absorption is a relevant route of exposure]. In a separate study, detectable levels of glyphosate were found in urine samples from farm families and non-farm families (Curwin *et al.*, 2007).

In accidental and deliberate intoxication cases involving ingestion of glyphosate-based formulations, glyphosate was readily detectable in the blood (Zouaoui *et al.*, 2013). After deliberate or accidental ingestion, one glyphosate-based formulation was found to be more lethal to humans than another (Sørensen & Gregersen, 1999). [Greater lethality was attributed to the presence of trimethylsulfonium counterion, which might facilitate greater absorption after oral exposure.]

Small amounts of glyphosate can be absorbed after dermal exposures in humans in vitro. For example, when an aqueous solution of 1% glyphosate was applied in an in-vitro human skin model, only 1.4% of the applied dose was absorbed through the skin. Glyphosate is typically formulated as an isopropylamine salt, and is dissolved in a water-based vehicle, while the

stratum corneum is a lipid-rich tissue (Wester *et al.*, 1991). In-vitro studies using human skin showed that percutaneous absorption of a glyphosate-based formulation was no more than 2% of the administered dose over a concentration range of 0.5–154 μg/cm² and a topical volume range of 0.014–0.14 mL/cm². In addition, very little glyphosate (≤ 0.05% of the administered dose) was sequestered in the stratum corneum after dermal application (Wester *et al.*, 1991).

In the human Caco-2 cell line, an in-vitro model of intestinal enterocytes, glyphosate (> 10 mg/mL) was shown to significantly disrupt barrier properties, leading to an increase in paracellular permeability (transport of substances that pass through the intercellular space between the cells) (Vasiluk *et al.*, 2005).

(b) Experimental systems

Three studies have been conducted to investigate the absorption of a single oral dose of glyphosate in rats (Brewster *et al.*, 1991; Chan & Mahler, 1992; EPA, 1993b).

In male Sprague-Dawley rats given [^{14}C]-labelled glyphosate (10 mg/kg bw), the majority of the radiolabel was associated with the gastrointestinal contents and small intestinal tissue 2 hours after administration (Brewster *et al.*, 1991). Approximately 35–40% of the administered dose was found to be absorbed from the gastrointestinal tract. Urinary and faecal routes of elimination were equally important. [The Working Group concluded that glyphosate is incompletely absorbed from the gastrointestinal tract after oral exposure in rats.]

In a study by the United States National Toxicology Programme (NTP) in Fisher 344 rats, 30% of the administered oral dose (5.6 mg/kg bw) was absorbed, as determined by urinary excretion data (Chan & Mahler, 1992). This finding was in accordance with the previously described study of oral exposure in rats (Brewster *et al.*, 1991).

In a study reviewed by the EPA, Sprague-Dawley rats were given an oral dose of glyphosate (10 mg/kg bw); 30% and 36% of the administered dose was absorbed in males and females, respectively (EPA, 1993b). At a dose that was ~10-fold higher (1000 mg/kg bw), oral absorption of glyphosate by the rats was slightly reduced.

In a 14-day feeding study in Wistar rats given glyphosate at dietary concentrations of up to 100 ppm, only ~15% of the administered dose was found to be absorbed (JMPR, 2006). In New Zealand White rabbits or lactating goats given glyphosate as single oral doses (6–9 mg/kg bw), a large percentage of the administered dose was recovered in the faeces [suggesting very poor gastrointestinal absorption of glyphosate in these animal models] (JMPR, 2006).

In monkeys given glyphosate by dermal application, percutaneous absorption was estimated to be between 1% and 2% of the administered dose (Wester *et al.*, 1991). Most of the administered dose was removed by surface washes of the exposed skin.

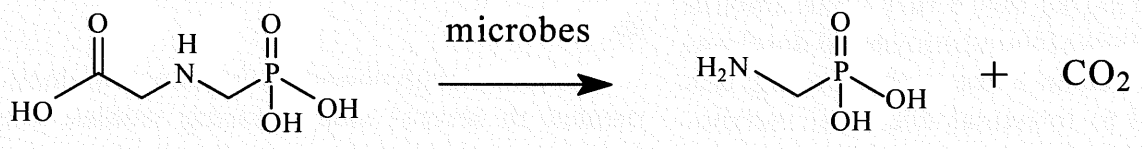
4.1.3 Distribution

(a) Humans

No data in humans on the distribution of glyphosate in systemic tissues other than blood were available to the Working Group. In cases of accidental or deliberate intoxication involving ingestion of glyphosate-based formulations, glyphosate was measured in blood. Mean blood concentrations of glyphosate were 61 mg/L and 4146 mg/L in mild-to-moderate cases of intoxication and in fatal cases, respectively (Zouaoui *et al.*, 2013).

One report, using optical spectroscopy and molecular modelling, indicated that glyphosate could bind to human serum albumin, mainly by hydrogen bonding; however, the fraction of glyphosate that might bind to serum proteins in blood was not actually measured (Yue *et al.*, 2008).

Fig. 4.1 Microbial metabolism of glyphosate to AMPA



Glyphosate is degraded to AMPA by microbial metabolism
Compiled by the Working Group

(b) Experimental systems

In Sprague-Dawley rats given a single oral dose of glyphosate (100 mg/kg bw), glyphosate concentrations in plasma reached peak levels, then declined slowly from day 1 to day 5 (Bernal *et al.*, 2010). The plasma data appeared to fit a one-compartment model with an elimination rate constant of k_{el} = 0.021 hour^{-1}. [The Working Group estimated the elimination half-life of glyphosate to be 33 hours.] Tissue levels of glyphosate were not determined in this study. In a study by Brewster *et al.* (1991), the tissue levels of glyphosate at 2, 6.3, 28, 96, and 168 hours in Sprague-Dawley rats given a single oral dose (10 mg/kg bw) declined rapidly. Tissues with the greatest amounts of detectable radiolabel (> 1% of the administered dose) were the small intestine, colon, kidney, and bone. Peak levels were reached in small intestine tissue and blood by 2 hours, while peak levels in other tissues occurred at 6.3 hours after dosing. After 7 days, the total body burden of [^{14}C]-labelled residues was ~1% of the administered dose, and was primarily associated with the bone (~1 ppm). In every tissue examined after administration of [^{14}C]-labelled glyphosate, essentially 100% of the radiolabel that was present in the tissue was unmetabolized parent glyphosate. Thus, essentially 100% of the body burden was parent compound, with no significant persistence of glyphosate after 7 days (Brewster *et al.*, 1991). In a 14-day feeding study in Wistar rats given diets containing glyphosate at 100 ppm, glyphosate reached steady-state levels in the blood by day 6 (JMPR, 2006). The tissue concentrations of glyphosate had the following rank order: kidneys > spleen > fat > liver. Tissue levels declined rapidly after cessation of exposure to glyphosate. A second study in rats given glyphosate (10 mg/kg bw per day, 14 days) followed by a single oral dose of [^{14}C]-glyphosate (at 10 mg/kg bw) showed that repeated dosing did not alter the tissue distribution of glyphosate (JMPR, 2006).

In rhesus monkeys, tissues harvested 7 days after dermal exposures to [^{14}C]-labelled glyphosate did not contain radiolabel at detectable levels (Wester *et al.*, 1991).

4.1.4 Metabolism and modulation of metabolic enzymes

(a) Metabolism

Glyphosate is degraded in the environment by soil microbes, primarily to AMPA and carbon dioxide (Fig. 4.1; Jacob *et al.*, 1988). A minor pathway for the degradation of glyphosate in bacteria (*Pseudomonas sp.* strain LBr) is via conversion to glycine (Jacob *et al.*, 1988). In a case of deliberate poisoning with a glyphosate-based formulation, small amounts of AMPA (15.1 µg/mL) were detectable in the blood (Motojyuku *et al.*, 2008) [suggesting that this pathway might also operate in humans]. In rats given a single high oral dose of glyphosate (100 mg/kg bw), small amounts of AMPA were detected in the plasma (Bernal *et al.*, 2010). In

male Sprague-Dawley rats given an oral dose of glyphosate (10 mg/kg bw), a very small amount of AMPA (< 0.04% of the administered dose) was detected in the colon 2 hours after dosing; this was attributed to intestinal microbial metabolism (Brewster *et al.*, 1991).

(b) Modulation of metabolic enzymes

(i) Humans

In human hepatic cell lines, treatment with one of four glyphosate-based formulations produced by the same company was shown to enhance CYP3A4 and CYP1A2 levels, while glutathione transferase levels were reduced (Gasnier *et al.*, 2010). [The Working Group noted that it was not clear whether the effects were caused by glyphosate alone or by the adjuvants contained in the formulation.]

(ii) Experimental systems

Exposure of Wistar rats to a glyphosate-based formulation significantly altered some hepatic xenobiotic enzyme activities (Larsen *et al.*, 2014). Liver microsomes obtained from male and female rats treated with the formulation exhibited ~50% reductions in cytochrome P450 (CYP450) content compared with control (untreated) rats. However, opposing effects were observed when assessing 7-ethoxycoumarin *O*-deethylase activity (7-ECOD, a non-specific CYP450 substrate). Female rats treated with the glyphosate-based formulation exhibited a 57% increase in hepatic microsomal 7-ECOD activity compared with controls, while male rats treated with the formulation exhibited a 58% decrease in this activity (Larsen *et al.*, 2014). [The Working Group noted that it was not clear whether the effects were caused by glyphosate alone or by adjuvants contained in the formulation.]

4.1.5 Excretion

(a) Humans

Excretion of glyphosate in humans was documented in several biomonitoring studies. For example, as part of the Farm Family Exposure Study, urinary concentrations of glyphosate were evaluated immediately before, during, and after glyphosate application in 48 farmers and their spouses and children (Acquavella *et al.*, 2004). Dermal contact with glyphosate during mixing, loading, and application was considered to be the main route of exposure in the study. On the day the herbicide was applied, 60% of the farmers had detectable levels of glyphosate in 24-hour composite urine samples, as did 4% of their spouses and 12% of children. For farmers, the geometric mean concentration was 3 µg/L, the maximum value was 233 µg/L, and the highest estimated systemic dose was 0.004 mg/kg bw (Acquavella *et al.*, 2004). In a separate study, detectable levels of glyphosate were excreted in the urine of members of farm families and of non-farm families, with geometric means ranging from 1.2 to 2.7 µg/L (Curwin *et al.*, 2007).

In a study of a rural population living near areas sprayed for drug eradication in Colombia (see Section 1.4.1, Table 1.5), mean urinary glyphosate concentrations were 7.6 µg/L (range, undetectable to 130 µg/L) (Varona *et al.*, 2009). AMPA was detected in 4% of urine samples (arithmetic mean, 1.6 µg/L; range, undetectable to 56 µg/L).

(b) Experimental systems

In an NTP study in Fisher 344 rats given a single oral dose of [^{14}C]-labelled glyphosate (5.6 or 56 mg/kg bw), it was shown that > 90% of the radiolabel was eliminated in the urine and faeces within 72 hours (Chan & Mahler, 1992). In Sprague-Dawley rats given [^{14}C]-labelled glyphosate at an oral dose of 10 or 1000 mg/kg bw, ~60–70% of the administered dose was excreted in the faeces, and the remainder in the urine (EPA,

1993b). By either route, most (98%) of the administered dose was excreted as unchanged parent compound. AMPA was the only metabolite found in the urine (0.2–0.3% of the administered dose) and faeces (0.2–0.4% of the administered dose). [The large amount of glyphosate excreted in the faeces is consistent with its poor oral absorption.] Less than 0.3% of the administered dose was expired as carbon dioxide.

In rhesus monkeys given glyphosate as an intravenous dose (9 or 93 μg), > 95% of the administered dose was excreted in the urine (Wester *et al.*, 1991). Nearly all the administered dose was eliminated within 24 hours. In contrast, in rhesus monkeys given glyphosate by dermal application (5400 μg/20 cm^2), only 2.2% of the administered dose was excreted in the urine within 7 days (Wester *et al.*, 1991).

Overall, systemically absorbed glyphosate is not metabolized efficiently, and is mainly excreted unchanged into the urine.

4.2 Mechanisms of carcinogenesis

4.2.1 Genetic and related effects

Glyphosate has been studied for genotoxic potential in a wide variety of assays. Studies carried out in exposed humans, in human cells in vitro, in other mammals in vivo and in vitro, and in non-mammalian systems in vivo and in vitro, respectively, are summarized in Table 4.1, Table 4.2, Table 4.3, Table 4.4, and Table 4.5. [A review article by Kier & Kirkland (2013) summarized the results of published articles and unpublished reports of studies pertaining to the genotoxicity of glyphosate and glyphosate formulations. A supplement to this report contained information on 66 unpublished regulatory studies. The conclusions and data tables for each individual study were included in the supplement; however, the primary study reports from which these data were extracted were not available to the Working Group. The information provided in the supplement was insufficient regarding topics such as details of statistical methods, choice of the highest dose tested, and verification of the target tissue exposure. The Working Group determined that the information in the supplement to Kier & Kirkland (2013) did not meet the criteria for data inclusion as laid out in the Preamble to the *IARC Monographs*, being neither "reports that have been published or accepted for publication in the openly available scientific literature" nor "data from governmental reports that are publicly available" (IARC, 2006). The review article and supplement were not considered further in the evaluation.]

(a) Humans

(i) Studies in exposed humans

See Table 4.1

In exposed individuals (n = 24) living in northern Ecuador in areas sprayed with a glyphosate-based formulation, a statistically significant increase in DNA damage (DNA strand breaks) was observed in blood cells collected 2 weeks to 2 months after spraying (Paz-y-Miño *et al.*, 2007). The same authors studied blood cells from individuals (n = 92) in 10 communities in Ecuador's northern border, who were sampled 2 years after the last aerial spraying with a herbicide mix containing glyphosate, and showed that their karyotypes were normal compared with those of a control group (Paz-y-Miño *et al.*, 2011).

Bolognesi *et al.* (2009) studied community residents (137 women of reproductive age and their 137 spouses) from five regions in Colombia. In three regions with exposures to glyphosate-based formulations from aerial spraying, blood samples were taken from the same individuals at three time-points (before spraying (baseline), 5 days after spraying and 4 months after spraying) to determine the frequency of micronucleus formation in lymphocytes. The baseline frequency of binucleated cells with micronuclei was significantly higher in subjects

from the three regions where there had been aerial spraying with glyphosate-formulations and in a fourth region with pesticide exposure (but not through aerial spraying), compared with a reference region (without use of pesticide). The frequency of micronucleus formation in peripheral blood lymphocytes was significantly increased, compared with baseline levels in the same individuals, after aerial spraying with glyphosate-based formulations in each of the three regions (see Table 4.1; Bolognesi *et al.*, 2009). Immediately after spraying, subjects who reported direct contact with the glyphosate-based spray showed a higher frequency of binucleated cells with micronuclei. However, the increase in frequency of micronucleus formation observed immediately after spraying was not consistent with the rates of application used in the regions, and there was no association between self-reported direct contact with pesticide sprays and frequency of binucleated cells with micronuclei. In subjects from one but not other regions, the frequency of binucleated cells with micronuclei was significantly decreased 4 months after spraying, compared with immediately after spraying.

(ii) Human cells in vitro

See Table 4.2

Glyphosate induced DNA strand breaks (as measured by the comet assay) in liver Hep-2 cells (Mañas *et al.*, 2009a), lymphocytes (Mladinic *et al.*, 2009b; Alvarez-Moya *et al.*, 2014), GM38 fibroblasts, the HT1080 fibrosarcoma cell line (Monroy *et al.*, 2005), and the TR146 buccal carcinoma line (Koller *et al.*, 2012). DNA strand breaks were induced by AMPA in Hep-2 cells (Mañas *et al.*, 2009b), and by a glyphosate-based formulation in the TR146 buccal carcinoma cell line (Koller *et al.*, 2012).

In human lymphocytes, AMPA (Mañas *et al.*, 2009b), but not glyphosate (Mañas *et al.*, 2009a), produced chromosomal aberrations. Glyphosate did not induce a concentration-related increase in micronucleus formation in human lymphocytes at levels estimated to correspond to occupational and residential exposure (Mladinic *et al.*, 2009a). Sister-chromatid exchange was induced by glyphosate (Bolognesi *et al.*, 1997), and by a glyphosate-based formulation (Vigfusson & Vyse, 1980; Bolognesi *et al.*, 1997) in human lymphocytes exposed in vitro.

(b) Experimental systems

(i) Non-human mammals in vivo

See Table 4.3

The ability of glyphosate or a glyphosate-based formulation to induce DNA adducts was studied in mice given a single intraperitoneal dose. Glyphosate induced DNA adducts (8-hydroxy deoxyguanosine) in the liver, but not in the kidney, while a glyphosate-based formulation caused a slight increase in DNA adducts in the kidney, but not in the liver (Bolognesi *et al.*, 1997). Peluso *et al.* (1998) showed that a glyphosate-based formulation (glyphosate, 30.4%), but not glyphosate alone, caused DNA adducts (as detected by ^{32}P-DNA post-labelling) in mouse liver and kidney. Glyphosate and a glyphosate-based formulation produced DNA strand breaks in the liver and kidney after a single intraperitoneal dose (Bolognesi *et al.*, 1997).

In mice given a single dose of glyphosate by gavage, no genotoxic effect was observed by the dominant lethal test (EPA, 1980a).

After a single intraperitoneal dose, no chromosomal aberrations were observed in the bone marrow of rats treated with glyphosate (Li & Long 1988), while chromosomal aberrations were increased in the bone marrow of mice given a glyphosate-based formulation (glyphosate isopropylamine salt, ~41%) (Prasad *et al.*, 2009). A single oral dose of a glyphosate-based formulation did not cause chromosomal aberrations in mice (Dimitrov *et al.*, 2006).

In mice treated by intraperitoneal injection, a single dose of glyphosate did not cause

Table 4.1 Genetic and related effects of glyphosate in exposed humans

Tissue	Cell type (if specified)	End-point	Test	Description of exposure and controls	Response[a]/ significance	Comments	Reference
Blood	NR	DNA damage	DNA strand breaks, comet assay	24 exposed individuals in northern Ecuador; areas sprayed with glyphosate-based formulation (sampling 2 weeks to 2 months after spraying); control group was 21 non-exposed individuals	+ $P < 0.001$		Paz-y-Miño *et al.* (2007)
Blood	NR	Chromosomal damage	Chromosomal aberrations	92 individuals in 10 communities, northern border of Ecuador; sampling 2 years after last aerial spraying with herbicide mix containing glyphosate); control group was 90 healthy individuals from several provinces without background of smoking or exposure to genotoxic substances (hydrocarbons, X-rays, or pesticides)	–	182 karyotypes were considered normal [Smoking status, NR]	Paz-y-Miño *et al.* (2011)
Blood	Lymphocytes	Chromosomal damage	Micronucleus formation	55 community residents, Nariño, Colombia; area with aerial glyphosate-based formulation spraying for coca and poppy eradication (glyphosate was tank-mixed with an adjuvant)	+ [$P < 0.001$]	*P* values for after spraying vs before spraying in the same individuals	Bolognesi *et al.* (2009)
Blood	Lymphocytes	Chromosomal damage	Micronucleus formation	53 community residents, Putumayo, Colombia; area with aerial glyphosate-based formulation spraying for coca and poppy eradication (glyphosate was tank-mixed with an adjuvant)	+ [$P = 0.01$]	*P* values for after spraying vs before spraying in the same individuals	Bolognesi *et al.* (2009)
Blood	Lymphocytes	Chromosomal damage	Micronucleus formation	27 community residents, Valle del Cauca, Colombia; area where glyphosate-based formulation was applied through aerial spraying for sugar-cane maturation (glyphosate was applied without adjuvant)	+ [$P < 0.001$]	*P* values for after spraying vs before spraying in the same individuals	Bolognesi *et al.* (2009)

[a] +, positive; –, negative
NR, not reported; vs, versus

Case 3:16-md-02741-VC Document 654-2 Filed 10/28/17 Page 93 of 138

micronucleus formation in the bone marrow (Rank *et al.*, 1993), although two daily doses did (Bolognesi *et al.*, 1997; Mañas *et al.*, 2009a). AMPA, the main metabolite of glyphosate, also produced micronucleus formation after two daily intraperitoneal doses (Mañas *et al.*, 2009b). Conflicting results for micronucleus induction were obtained in mice exposed intraperitoneally to a glyphosate-based formulation. A single dose of the formulation at up to 200 mg/kg bw did not induce micronucleus formation in the bone marrow in one study (Rank *et al.* 1993), while it did increase micronucleus formation at 25 mg/kg bw in another study (Prasad *et al.*, 2009). After two daily intraperitoneal doses, a glyphosate-based formulation did not induce micronucleus formation at up to 200 mg/kg bw according to Grisolia (2002), while Bolognesi *et al.* (1997) showed that the formulation did induce micronucleus formation at 450 mg/kg bw. In mice given a single oral dose of a glyphosate-based formulation at 1080 mg/kg bw, no induction of micronuclei was observed (Dimitrov *et al.*, 2006).

(ii) Non-human mammalian cells in vitro

See Table 4.4

Glyphosate did not induce unscheduled DNA synthesis in rat primary hepatocytes, or *Hprt* mutation (with or without metabolic activation) in Chinese hamster ovary cells (Li & Long, 1988).

In bovine lymphocytes, chromosomal aberrations were induced by glyphosate in one study (Lioi *et al.*, 1998), but not by a glyphosate formulation in another study (Siviková & Dianovský, 2006). Roustan *et al.* (2014) demonstrated, in the CHO-K1 ovary cell line, that glyphosate induced micronucleus formation only in the presence of metabolic activation, while AMPA induced micronucleus formation both with and without metabolic activation. Sister-chromatid exchange was observed in bovine lymphocytes exposed to glyphosate (Lioi *et al.*, 1998) or a glyphosate formulation (in the absence but not the presence of metabolic activation) (Siviková & Dianovský, 2006).

(iii) Non-mammalian systems in vivo

See Table 4.5

Fish and other species

In fish, glyphosate produced DNA strand breaks in the comet assay in sábalo (Moreno *et al.*, 2014), European eel (Guilherme *et al.*, 2012b), zebrafish (Lopes *et al.*, 2014), and Nile tilapia (Alvarez-Moya *et al.*, 2014). AMPA also induced DNA strand breaks in the comet assay in European eel (Guilherme *et al.*, 2014b). A glyphosate-based formulation produced DNA strand breaks in numerous fish species, such as European eel (Guilherme *et al.*, 2010, 2012b, 2014a; Marques *et al.*, 2014, 2015), sábalo (Cavalcante *et al.*, 2008; Moreno *et al.*, 2014), guppy (De Souza Filho *et al.*, 2013), bloch (Nwani *et al.*, 2013), neotropical fish *Corydoras paleatus* (de Castilhos Ghisi & Cestari, 2013), carp (Gholami-Seyedkolaei *et al.*, 2013), and goldfish (Cavaş & Könen, 2007).

AMPA, the main metabolite of glyphosate, induced erythrocytic nuclear abnormalities (kidney-shaped and lobed nuclei, binucleate or segmented nuclei and micronuclei) in European eel (Guilherme *et al.*, 2014b). Micronucleus formation was induced by different glyphosate-based formulations in various fish (Grisolia, 2002; Cavaş & Könen, 2007; De Souza Filho *et al.*, 2013; Vera-Candioti *et al.*, 2013).

Glyphosate-based formulations induced DNA strand breaks in other species, including caiman (Poletta *et al.*, 2009), frog (Meza-Joya *et al.*, 2013), tadpoles (Clements *et al.*, 1997), and snail (Mohamed, 2011), but not in oyster (Akcha *et al.*, 2012), clam (dos Santos & Martinez, 2014), and mussel glochidia (Conners & Black, 2004). In earthworms, one glyphosate-based formulation induced DNA strand breaks while two others did not (Piola *et al.*, 2013; Muangphra *et al.*, 2014), highlighting the potential importance of components other than the active ingredient in the formulation.

Table 4.2 Genetic and related effects of glyphosate, AMPA, and glyphosate-based formulations in human cells in vitro

Tissue, cell line	End-point	Test	Results[a]		Dose (LED or HID)	Comments	Reference
			Without metabolic activation	With metabolic activation			
Glyphosate							
Liver Hep-2	DNA damage	DNA strand breaks, comet assay	+	NT	3 mM [507.2 µg/mL]	$P < 0.01$; dose–response relationship ($r \geq 0.90$; $P < 0.05$)	Mañas et al. (2009a)
Lymphocytes	DNA damage	DNA strand breaks, standard and hOGG1 modified comet assay	+	+	3.5 µg/mL	With the hOGG1 modified comet assay, + S9, the increase was significant ($P < 0.01$) only at the highest dose tested (580 µg/mL)	Mladinic *et al.* (2009b)
Lymphocytes	DNA damage	DNA strand breaks, comet assay	+	NT	0.0007 mM [0.12 µg/mL]	$P \leq 0.01$	Alvarez-Moya *et al.* (2014)
Fibroblast GM 38	DNA damage	DNA strand breaks, comet assay	+	NT	4 mM [676 µg/mL]	$P < 0.001$	Monroy *et al.* (2005)
Fibroblast GM 5757	DNA damage	DNA strand breaks, comet assay	(+)	NT	75 mM [12 680 µg/mL]	Glyphosate (ineffective alone, data NR) increased strand breaks induced by H_2O_2 (40 or 50 µM) ($P < 0.004$ vs H_2O_2 alone)	Lueken *et al.* (2004)
Fibrosarcoma HT1080	DNA damage	DNA strand breaks, comet assay	+	NT	4.75 mM [803 µg/mL]	$P < 0.001$	Monroy *et al.* (2005)
Buccal carcinoma TR146	DNA damage	DNA strand breaks, SCGE assay	+	NT	20 µg/mL	Dose-dependent increase ($P \leq 0.05$)	Koller *et al.* (2012)
Lymphocytes	Chromosomal damage	Chromosomal aberrations	–	NT	6 mM [1015 µg/mL]		Mañas *et al.* (2009a)
Lymphocytes	Chromosomal damage	Micronucleus formation	–	(+)	580 µg/mL	$P < 0.01$ at the highest exposure + S9 No concentration-related increase in micronuclei containing the centromere signal (C+)	Mladinic *et al.* (2009a)

Case 3:16-md-02741-VC Document 654-2 Filed 10/28/17 Page 95 of 138

Table 4.2 (continued)

Tissue, cell line	End-point	Test	Results[a]		Dose (LED or HID)	Comments	Reference
			Without metabolic activation	With metabolic activation			
Lymphocytes	Chromosomal damage	Sister-chromatid exchange	+	NT	1000 µg/mL	$P < 0.05$	Bolognesi *et al.* (1997)
AMPA							
Liver Hep-2	DNA damage	DNA strand breaks, comet assay	+	NT	4.5 mM [500 µg/mL]	$P < 0.05$ at 4.5 mM; $P < 0.01$ at up to 7.5 mM Dose–response relationship ($r \geq 0.90$; $P < 0.05$)	Mañas *et al.* (2009b)
Lymphocytes	Chromosomal damage	Chromosomal aberrations	+	NT	1.8 mM [200 µg/mL]	$P < 0.05$	Mañas *et al.* (2009b)
Glyphosate-based formulations							
Liver HepG2	DNA damage	DNA strand breaks, comet assay	(+)	NT	5 ppm	Glyphosate, 400 g/L Dose-dependent increase; greatest increase at 10 ppm Statistical analysis, NR	Gasnier *et al.* (2009)
Buccal carcinoma TR146	DNA damage	DNA strand breaks, SCGE assay	+	NT	20 µg/mL	Glyphosate acid, 450g/L Dose-dependent increase ($P \leq 0.05$)	Koller *et al.* (2012)
Lymphocytes	Chromosomal damage	Sister-chromatid exchange	+	NT	250 µg/mL	$P < 0.001$ No growth at 25 mg/mL	Vigfusson & Vyse (1980)
Lymphocytes	Chromosomal damage	Sister-chromatid exchange	+	NT	100 µg/mL	Glyphosate, 30.4% $P < 0.05$	Bolognesi *et al.* (1997)

[a] +, positive; -, negative; (+) or (-) positive/negative in a study with limited quality

AMPA, aminomethyl phosphonic acid; HID, highest ineffective dose; hOGG1, human 8-hydroxyguanosine DNA-glycosylase; LED, lowest effective dose; NR, not reported; NT, not tested; S9, 9000 × *g* supernatant; SCGE, single cell gel electrophoresis; vs, versus

Case 3:16-md-02741-VC Document 654-2 Filed 10/28/17 Page 96 of 138

Micronucleus formation was induced by a glyphosate-based formulation (glyphosate, 36%) in earthworms (Muangphra *et al.*, 2014), and by a different glyphosate-based formulation in caiman (Poletta *et al.*, 2009, 2011), and frog (Yadav *et al.*, 2013).

Insects

In standard *Drosophila melanogaster*, glyphosate induced mutation in the test for somatic mutation and recombination, but not in a cross of flies characterized by an increased capacity for CYP450-dependent bioactivation (Kaya *et al.*, 2000). A glyphosate-based formulation also caused sex-linked recessive lethal mutations in *Drosophila* (Kale *et al.*, 1995).

Plants

In plants, glyphosate produced DNA damage in *Tradescantia* in the comet assay (Alvarez-Moya *et al.*, 2011). Chromosomal aberration was induced after exposure to glyphosate in fenugreek (Siddiqui *et al.*, 2012), and in onion in one study (Frescura *et al.*, 2013), but not in another (Rank *et al.*, 1993). A glyphosate-based formulation also induced chromosomal aberration in barley roots (Truta *et al.*, 2011) and onion (Rank *et al.*, 1993), but not in *Crepis capillaris* (hawksbeard) (Dimitrov *et al.*, 2006). Micronucleus formation was not induced by glyphosate in *Vicia faba* bean (De Marco *et al.*, 1992) or by a glyphosate-based formulation in *Crepis capillaris* (Dimitrov *et al.*, 2006).

(iv) Non-mammalian systems in vitro

See Table 4.6

Glyphosate induced DNA strand breaks in erythrocytes of tilapia fish, as demonstrated by comet assay (Alvarez-Moya *et al.*, 2014).

Glyphosate did not induce mutation in *Bacillus subtillis*, *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98, and TA100, or in *Escherichia coli* WP2, with or without metabolic activation (Li & Long, 1988). However, Rank *et al.* (1993) demonstrated that a glyphosate-based formulation was mutagenic in *S. typhimurium* TA98 in the absence of metabolic activation, and in *S. typhimurium* TA100 in the presence of metabolic activation.

4.2.2 Receptor-mediated mechanisms

(a) Sex-hormone pathway disruption

(i) Humans

Studies in exposed humans

No data were available to the Working Group.

Human cells in vitro

In hormone-dependent T47D breast cancer cells, the proliferative effects of glyphosate (10^{-6} to 1 μM) (see Section 4.2.4) and those of 17β-estradiol (the positive control) were mitigated by the estrogen receptor antagonist, ICI 182780; the proliferative effect of glyphosate was completely abrogated by the antagonist at a concentration of 10 nM (Thongprakaisang *et al.*, 2013). Glyphosate also induced activation of the estrogen response element (ERE) in T47D breast cancer cells that were stably transfected with a triplet ERE-promoter-luciferase reporter gene construct. Incubation with ICI 182780 at 10 nM eliminated the response. When the transfected cells were incubated with both 17β-estradiol and glyphosate, the effect of 17β-estradiol was reduced and glyphosate behaved as an estrogen antagonist. After 6 hours of incubation, glyphosate increased levels of estrogen receptors ERα and ERβ in a dose-dependent manner in T47D cells; after 24 hours, only ERβ levels were increased and only at the highest dose of glyphosate. [These findings suggested that the proliferative effects of glyphosate on T47D cells are mediated by ER.]

In human hepatocarcinoma HepG2 cells, four glyphosate-based formulations produced by the same company had a marked effect on the activity and transcription of aromatase, while glyphosate alone differed from controls, but not significantly so (Gasnier *et al.*, 2009).

Case 3:16-md-02741-VC Document 654-2 Filed 10/28/17 Page 98 of 138

Table 4.3 Genetic and related effects of glyphosate, AMPA, and glyphosate-based formulations in non-human mammals in vivo

Species, strain (sex)	Tissue	End-point	Test	Results	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Glyphosate								
Mouse, Swiss CD1 (M)	Liver	DNA damage	DNA adducts, 8-OHdG by LC/UV	+	300 mg/kg bw	i.p.; 1×; sampled after 8 and 24 h	Single dose tested only $P < 0.05$ after 24 h	Bolognesi *et al.* (1997)
Mouse, Swiss CD1 (M)	Kidney	DNA damage	DNA adducts, 8-OHdG by LC/UV	–	300 mg/kg bw	i.p.; 1×; sampled after 8 and 24 h	Single dose tested only	Bolognesi *et al.* (1997)
Mouse, Swiss CD1 (M, F)	Kidney	DNA damage	DNA adducts, ^{32}P-DNA post labelling	–	270 mg/kg bw	i.p.; 1 ×; sampled after 24 h	Glyphosate isopropylammonium salt	Peluso *et al.* (1998)
Mouse, Swiss CD1 (M, F)	Liver	DNA damage	DNA adducts, ^{32}P-DNA post labelling	–	270 mg/kg bw	i.p.; 1 ×; sampled after 24 h	Glyphosate isopropylammonium salt	Peluso *et al.* (1998)
Mouse, Swiss CD1 (M)	Liver	DNA damage	DNA strand breaks, alkaline elution assay	+	300 mg/kg bw	i.p.; 1 ×; sampled after 4 and 24 h	Single dose tested only $P < 0.05$ after 4 h	Bolognesi *et al.* (1997)
Mouse, Swiss CD1 (M)	Kidney	DNA damage	DNA strand breaks, alkaline elution assay	+	300 mg/kg bw	i.p.; 1 ×; sampled after 4 and 24 h	Single dose tested only $P < 0.05$ after 4 h	Bolognesi *et al.* (1997)
Mouse, CD-1 (M)	Uterus after mating	Mutation	Dominant lethal test	–	2000 mg/kg bw	Oral gavage; 1 ×	Proportion of early resorptions evaluated after mating of non-treated females with glyphosate-treated male mice	EPA (1980)
Rat, Sprague-Dawley (M, F)	Bone marrow	Chromosomal damage	Chromosomal aberrations	–	1000 mg/kg bw	i.p.; 1 ×; sampled after 6, 12 and 24 h	Single dose tested only	Li & Long (1988)
Mouse, NMRI-bom (M, F)	Bone marrow (PCE)	Chromosomal damage	Micronucleus formation	–	200 mg/kg bw	i.p.; 1 ×; sampled after 24 and 48 h	Glyphosate isopropylamine salt	Rank *et al.* (1993)
Mouse, Swiss CD1 (M)	Bone marrow (PCE)	Chromosomal damage	Micronucleus formation	+	300 mg/kg bw	i.p.; 2 × 150 mg/kg bw with 24 h interval; sampled 6 or 24 h after the last injection	Single dose tested only $P < 0.05$ after 24 h	Bolognesi *et al.* (1997)

Table 4.3 (continued)

Species, strain (sex)	Tissue	End-point	Test	Results	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Mouse, Balb C (M, F)	Bone marrow (PCE)	Chromosomal damage	Micronucleus formation	+	400 mg/kg bw	i.p.; one injection per 24 h, 2 × 200, sampled 24 h after the last injection	$P < 0.01$ at the highest dose (400 mg/kg bw)	Mañas *et al.* (2009a)
AMPA								
Mouse, Balb C (M, F)	Bone marrow (PCE)	Chromosomal damage	Micronucleus formation	+	200 mg/kg bw	i.p.; one injection per 24 h, 2 × 100, sampled 24 h after the last injection	$P < 0.01$ at the lowest dose (200 mg/kg bw)	Mañas *et al.* (2009b)
Glyphosate-based formulations								
Mouse, Swiss CD1 (M)	Liver	DNA damage	DNA adducts, 8-OHdG by LC/UV	–	~300 mg/kg bw	i.p.; 1 ×, sampled after 8 and 24 h	Glyphosate, 30.4% Single dose tested only	Bolognesi *et al.* (1997)
Mouse, Swiss CD1 (M)	Kidney	DNA damage	DNA adducts, 8-OHdG by LC/UV	+	~300 mg/kg bw	i.p.; 1 ×, sampled after 8 and 24 h	Glyphosate, 30.4% Single dose tested only $P < 0.05$	Bolognesi *et al.* (1997)
Mouse, Swiss CD1 (M, F)	Kidney	DNA damage	DNA adducts, ^{32}P-DNA post labelling	+	400 mg/kg bw	i.p.; 1 ×; sampled after 24 h	Glyphosate isopropylammonium salt, 30.4%	Peluso *et al.* (1998)
Mouse, Swiss CD1 (M, F)	Liver	DNA damage	DNA adducts, ^{32}P-DNA post labelling	+	400 mg/kg bw	i.p.; 1 ×; sampled after 24 h	Glyphosate isopropylammonium salt, 30.4%	Peluso *et al.* (1998)
Mouse, Swiss CD1 (M)	Liver	DNA damage	DNA strand breaks, alkaline elution assay	+	~300 mg/kg bw	i.p.; 1 ×; sampled after 4 and 24 h	Glyphosate, 30.4% Single dose tested only $P < 0.05$ only after 4 h	Bolognesi *et al.* (1997)
Mouse, Swiss CD1 (M)	Kidney	DNA damage	DNA strand breaks, alkaline elution assay	+	~300 mg/kg bw	i.p.; 1 ×; sampled after 4 and 24 h	Glyphosate, 30.4% Single dose tested only $P < 0.05$ only after 4 h	Bolognesi *et al.* (1997)
Mouse, C57BL (M)	Bone marrow (PCE)	Chromosomal damage	Chromosomal aberrations	–	1080 mg/kg bw	p.o. in distilled water; 1 ×; sampled after 6, 24, 48, 72, 96 and 120 h	Single dose tested only	Dimitrov *et al.* (2006)

Case 3:16-md-02741-VC Document 654-2 Filed 10/28/17 Page 99 of 138

Table 4.3 (continued)

Species, strain (sex)	Tissue	End-point	Test	Results	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Mouse, Swiss albino (M)	Bone marrow	Chromosomal damage	Chromosomal aberrations	+	25 mg/kg bw	i.p.; 1 ×; sampled after 24, 48 and 72 h	Glyphosate isopropylamine salt, > 41% The percentage of aberrant cells was increased vs control in a dose- and time-dependent manner ($P < 0.05$)	Prasad *et al.* (2009)
Mouse, NMRI-bom (M, F)	Bone marrow (PCE)	Chromosomal damage	Micronucleus formation	–	200 mg/kg bw	i.p.; 1 ×; sampled after 24 h	Glyphosate isopropylammonium salt, 480 g/L The percentage of PCE decreased	Rank *et al.* (1993)
Mouse, Swiss (M, F)	Bone marrow (PCE)	Chromosomal damage	Micronucleus formation	–	200 mg/kg bw	i.p.; 2 × within 24 h interval and sampled 24 h after the last injection	Glyphosate isopropylammonium salt, 480 g/L	Grisolia (2002)
Mouse, Swiss albino (M)	Bone marrow (PCE)	Chromosomal damage	Micronucleus formation	+	25 mg/kg bw	i.p.; 1 × ; sampled after 24, 48 and 72 h	Glyphosate isopropylamine salt, > 41% Significant induction of micronuclei vs control at both doses and all times ($P < 0.05$)	Prasad *et al.* (2009)
Mouse, Swiss CD1 (M)	Bone marrow (PCE)	Chromosomal damage	Micronucleus formation	+	450 mg/kg bw	i.p.; 2 × 225 mg/kg with 24 h interval; sampled 6 or 24 h after the last injection	Glyphosate, 30.4% Single dose tested only $P < 0.05$ after 6 h and 24 h	Bolognesi *et al.* (1997)
Mouse, C57BL (M)	Bone marrow	Chromosomal damage	Micronucleus formation	–	1080 mg/kg bw	p.o. in distilled water; 1 × ; sampled after 24, 48, 72, 96 and 120 h	Single dose tested only	Dimitrov *et al.* (2006)

[a] +, positive; -, negative; (+) or (-) positive/negative in a study with limited quality

bw, body weight; F, female; h, hour; HID, highest effective dose; i.p., intraperitoneal; LC, liquid chromatography; LED, lowest effective dose; M, male; PCE, polychromatic erythrocytes; p.o., oral; 8-OHdG, 8-hydroxydeoxyguanosine; UV, ultraviolet

Case 3:16-md-02741-VC Document 654-2 Filed 10/28/17 Page 100 of 138

Table 4.4 Genetic and related effects of glyphosate, AMPA, and glyphosate-based formulations in non-human mammalian cells in vitro

Species	Tissue, cell line	End-point	Test	Results[a]		Dose (LEC or HIC)	Comments	Reference
				Without metabolic activation	With metabolic activation			
Glyphosate								
Rat, Fisher F334	Hepatocytes	DNA damage	Unscheduled DNA synthesis	–	NT	125 µg/mL		Li & Long (1988)
Hamster, Chinese	CHO-K_1BH_4 ovary, cell line	Mutation	*Hprt* mutation	–	–	22 500 µg/mL		Li & Long (1988)
Bovine	Lymphocytes	Chromosomal damage	Chromosomal aberrations	+	NT	17 µM [3 µg/mL]	$P < 0.05$	Lioi *et al.* (1998)
Hamster, Chinese	CHO-K1 ovary cell line	Chromosomal damage	Micronucleus formation	–	+	10 µg/mL	$P \leq 0.001$, in the dark +S9 Negative –S9 in the dark or with light irradiation	Roustan *et al.* (2014)
Bovine	Lymphocytes	Chromosomal damage	Sister-chromatid exchange	+	NT	17 µM [3 µg/mL]	$P < 0.05$	Lioi *et al.* (1998)
AMPA								
Hamster, Chinese	CHO-K1 ovary cell line	Chromosomal damage	Micronucleus formation	+	+	0.01 µg/mL	$P \leq 0.05$, in the dark –S9 Highest increase was observed at very low dose (0.0005 µg/mL) –S9 but with light-irradiation ($P < 0.01$)	Roustan *et al.* (2014)
Glyphosate-based formulations								
Bovine	Lymphocytes	Chromosomal damage	Chromosomal aberrations	–	NT	1120 µM [190 µg/mL]	Glyphosate, 62%	Siviková & Dianovský (2006)
Bovine	Lymphocytes	Chromosomal damage	Sister-chromatid exchange	+	–	56 µM [9.5 µg/mL]	Glyphosate, 62% Time of exposure, 24 h $P < 0.01$, –S9, at ≥ 56 µM	Siviková & Dianovský (2006)

[a] +, positive; –, negative; (+), weakly positive

AMPA, aminomethyl phosphonic acid; HIC, highest ineffective concentration; *Hprt*, hypoxanthine guanine phosphoribosyl transferase gene; LEC, lowest effective concentration; NT, not tested

Case 3:16-md-02741-VC Document 654-2 Filed 10/28/17 Page 101 of 138

Case 3:16-md-02741-VC Document 654-2 Filed 10/28/17 Page 102 of 138

Table 4.5 Genetic and related effects of glyphosate, AMPA, and glyphosate-based formulations in non-mammalian systems in vivo

Phylogenetic class	Species, strain, tissue	End-point	Test	Results[a]	Dose (LED or HID)	Comments	Reference
Glyphosate							
Fish	*Prochilodus lineatus* (sábalo), erythrocytes and gill cells	DNA damage	DNA strand breaks, comet assay	+	0.48 mg/L	Time of exposure 6, 24, and 96 h For erythrocytes, $P = 0.01$ after 6 h, and $P = 0.014$ after 96 h; no significant increase after 24 h For gill cells, $P = 0.02$ only after 6 h at 2.4 mg/L	Moreno *et al.* (2014)
Fish	*Anguilla anguilla* L. (European eel), blood cells	DNA damage	DNA strand breaks, comet assay	+	0.0179 mg/L	Time of exposure 1 and 3 days $P < 0.05$	Guilherme *et al.* (2012b)
Fish	*Danio rerio* (zebrafish), sperm	DNA damage	DNA strand breaks, acridine orange method	+	10 mg/L	After 96 h, DNA integrity was 78.3 ± 3.5%, significantly reduced from control (94.7 ± 0.9%) and 5 mg/L (92.6 ± 1.9%), ($P < 0.05$)	Lopes *et al.* (2014)
Fish	*Oreochromis niloticus* (Nile tilapia) branchial erythrocytes	DNA damage	DNA strand breaks, comet assay	+	7 µM [1.2 mg/L]	Time of exposure, 10 days $P < 0.001$ with concentrations ≥ 7 µM	Alvarez-Moya *et al.* (2014)
Oyster	Oyster spermatozoa	DNA damage	DNA strand breaks, comet assay	–	0.005 mg/L	Time of exposure, 1 h	Akcha *et al.* (2012)
Insect	*Drosophila* standard cross	Mutation	SMART	+	1 mM [0.169 mg/L]	Purity, 96% Increased frequency of small single spots (≥ 1 mM) and total spots (≥ 2 mM) $P = 0.05$	Kaya *et al.* (2000)
Insect	*Drosophila melanogaster*, high bioactivation cross	Mutation	SMART	–	10 mM [1.69 mg/L]	Purity, 96%	Kaya *et al.* (2000)

Table 4.5 (continued)

Phylogenetic class	Species, strain, tissue	End-point	Test	Results[a]	Dose (LED or HID)	Comments	Reference
Plant systems	*Tradescantia* clone 4430 (spiderworts), staminal hair nuclei	DNA damage	DNA strand breaks, comet assay	+	0.0007 mM [0.12 µg/mL]	Glyphosate isopropylamine salt $P < 0.01$ for directly exposed nuclei (dose-dependent increase) and plants	Alvarez-Moya *et al.* (2011)
Plant systems	*Allium cepa* (onion)	Chromosomal damage	Chromosomal aberrations	+	3%	Single dose tested only Partial but significant reversal with distilled water	Frescura *et al.* (2013)
Plant systems	*Allium cepa* (onion)	Chromosomal damage	Chromosomal aberrations	–	2.88 µg/mL	Glyphosate isopropylamine	Rank *et al.* (1993)
Plant systems	*Trigonella foenum-graecum* L. (fenugreek)	Chromosomal damage	Chromosomal aberrations	+	0.2%	$P < 0.001$; positive dose–response relationship	Siddiqui *et al.* (2012)
Plant systems	*Vicia faba* (bean)	Chromosomal damage	Micronucleus formation	–	1400 ppm (1400 µg/g of soil)	Tested with two types of soil, but not without soil	De Marco *et al.* (1992)
AMPA							
Fish	*Anguilla anguilla* L. (European eel)	DNA damage	DNA strand breaks, comet assay	+	0.0118 mg/L	Time of exposure, 1 and 3 days $P < 0.05$ after 1 day of exposure	Guilherme *et al.* (2014b)
Fish	*Anguilla anguilla* L. (European eel)	Chromosomal damage	Other (ENA)	+	0.0236 mg/L	$P < 0.05$ only at highest dose after 3 day exposure (not after 1 day)	Guilherme *et al.* (2014b)
Glyphosate-based formulations							
Fish	*Anguilla anguilla* L. (European eel), blood cells	DNA damage	DNA strand breaks, comet assay	+	0.058 mg/L	$P < 0.05$ Positive dose–response relationship	Guilherme *et al.* (2010)
Fish	*Anguilla anguilla* L. (European eel), blood cells	DNA damage	DNA strand breaks, comet assay improved with the DNA-lesion-specific FPG and Endo III	+	0.058 mg/L	Glyphosate-based formulation, 30.8% Time of exposure, 1 and 3 days With FPG, $P < 0.05$; with comet assay alone, $P < 0.05$ at 116 µg/L	Guilherme *et al.* (2012b)

Case 3:16-md-02741-VC Document 654-2 Filed 10/28/17 Page 103 of 138

Case 3:16-md-02741-VC Document 654-2 Filed 10/28/17 Page 104 of 138

Table 4.5 (continued)

Phylogenetic class	Species, strain, tissue	End-point	Test	Results[a]	Dose (LED or HID)	Comments	Reference
Fish	*Anguilla anguilla* L. (European eel), blood cells	DNA damage	DNA strand breaks, comet assay improved with the DNA-lesion-specific FPG and Endo III	+	0.116 mg/L	Single dose tested only Time of exposure, 3 days; recovery from non-specific DNA damage, but not oxidative DNA damage, 14 days after exposure $P < 0.05$	Guilherme *et al.* (2014a)
Fish	*Anguilla anguilla* L. (European eel), liver	DNA damage	DNA strand breaks, comet assay improved with the DNA-lesion-specific FPG and Endo III	+	0.058 mg/L	Glyphosate-based formulation, 485 g/L Time of exposure, 3 days $P < 0.05$	Marques *et al.* (2014, 2015)
Fish	*Prochilodus lineatus* (sábalo), erythrocytes and bronchial cells	DNA damage	DNA strand breaks, comet assay	+	10 mg/L	Single dose tested only, for 6, 24, and 96 h $P < 0.05$ for both erythrocytes and bronchial cells	Cavalcante *et al.* (2008)
Fish	*Prochilodus lineatus* (sábalo), erythrocytes and gill cells	DNA damage	DNA strand breaks, comet assay	+	1 mg/L	Glyphosate-based formulation, 480 g/L Time of exposure, 6, 24 and 96 h $P < 0.001$ after 24 and 96 h in erythrocytes and 24 h in gill cells	Moreno *et al.* (2014)
Fish	*Poecilia reticulata* (guppy) gill erythrocytes	DNA damage	DNA strand breaks, comet assay	+	2.83 µL/L [1.833 mg/L]	Glyphosate, 64.8%, m/v (648 g/L) $P < 0.05$	De Souza Filho *et al.* (2013)
Fish	*Channa punctatus* (bloch), blood and gill cells	DNA damage	DNA strand breaks, comet assay	+	3.25 mg/L	Exposure continued for 35 days; blood and gill cells collected on day 1, 7, 14, 21, 28 and 35 $P < 0.01$, for blood and gill cells; DNA damage increased with time and concentration	Nwani *et al.* (2013)

Table 4.5 (continued)

Phylogenetic class	Species, strain, tissue	End-point	Test	Results[a]	Dose (LED or HID)	Comments	Reference
Fish	*Corydoras paleatus* (blue leopard corydoras, mottled corydoras and peppered catfish), blood and hepatic cells	DNA damage	DNA strand breaks, comet assay	+	0.0067 mg/L	Glyphosate, 48% (corresponding to 3.20 µg/L) Single dose tested only, for 3, 6, and 9 days $P < 0.01$, in blood and in liver cells	de Castilhos Ghisi & Cestari (2013)
Fish	*Cyprinus carpio* Linnaeus (carp), erythrocytes	DNA damage	DNA strand breaks, comet assay	+	2 mg/L (10% LC_{50}, 96 h)	Glyphosate, equivalent to 360 g/L Single dose tested only, for 16 days $P < 0.01$	Gholami-Seyedkolaei *et al.* (2013)
Fish	*Carassius auratus* (goldfish), erythrocytes	DNA damage	DNA strand breaks, comet assay	+	5 ppm	Glyphosate equivalent to 360 g/L Time of exposure, 2, 4 and 6 days After 48 h: $P < 0.05$ (5 mg/L) and $P < 0.001$ (10 and 15 mg/L)	Cavaş & Könen (2007)
Fish	*Prochilodus lineatus* (sábalo) erythrocytes	Chromosomal damage	Micronucleus formation	–	10 mg/L	Single dose tested only, for 6, 24, and 96 h Nuclear abnormalities (lobed nuclei, segmented nuclei and kidney-shaped nuclei)	Cavalcante *et al.* (2008)
Fish	*Corydoras paleatus* (blue leopard corydoras, mottled corydoras and peppered catfish), blood and hepatic cells	Chromosomal damage	Micronucleus formation	–	0.0067 mg/L	Glyphosate, 48% (corresponding to 3.20 µg/L) Single dose tested only, for 3, 6 and 9 days	de Castilhos Ghisi & Cestari (2013)

Case 3:16-md-02741-VC Document 654-2 Filed 10/28/17 Page 105 of 138

Case 3:16-md-02741-VC Document 654-2 Filed 10/28/17 Page 106 of 138

Table 4.5 (continued)

Phylogenetic class	Species, strain, tissue	End-point	Test	Results[a]	Dose (LED or HID)	Comments	Reference
Fish	*Tilapia rendalli* (redbreast tilapia) blood erythrocytes	Chromosomal damage	Micronucleus formation	+	42 mg/kg bw	Glyphosate, 480 g/L Increased frequency of micronucleus formation vs control ($P < 0.05$) in blood samples collected 4 days after a single intra-abdominal injection of 42, 85, or 170 mg/kg bw	Grisolia (2002)
Fish	*Carassius auratus* (goldfish), erythrocytes	Chromosomal damage	Micronucleus formation	+	5 ppm	Glyphosate equivalent to 360 g/L Time of exposure, 2, 4 and 6 days Statistically significant differences: 96 h ($P < 0.05$); 144 h ($P < 0.01$)	Cavaş & Könen (2007)
Fish	*Poecilia reticulata* (guppy) gill erythrocytes	Chromosomal damage	Micronucleus formation, ENA	+	1.41 µL/L [0.914 mg/L]	Glyphosate, 64.8%, m/v (648 g/L) Micronucleus formation, $P < 0.01$ Other nuclear abnormalities, $P < 0.05$ at 1.41 to 5.65 µL/L; concentration-dependent ($r^2 = 0.99$)	De Souza Filho *et al.* (2013)
Fish	*Cnesterodon decemmaculatus* (Jenyns, 1842) peripheral blood erythrocytes	Chromosomal damage	Micronucleus formation	+	3.9 mg/L	Glyphosate, 48% Time of exposure, 48 and 96 h $P < 0.05$, with 3.9 and 7.8 mg/L for 48 and 96 h	Vera-Candioti *et al.* (2013)
Fish	*Cnesterodon decemmaculatus* (Jenyns, 1842) peripheral blood erythrocytes	Chromosomal damage	Micronucleus formation	+	22.9 mg/L	Glyphosate, 48% Time of exposure, 48 and 96 h $P < 0.01$, with 22.9 and 45.9 mg/L, and $P < 0.05$ at 68.8 mg/L, for 96 h	Vera-Candioti *et al.* (2013)

Table 4.5 (continued)

Phylogenetic class	Species, strain, tissue	End-point	Test	Results[a]	Dose (LED or HID)	Comments	Reference
Fish	*Prochilodus lineatus* (sábalo) erythrocytes	Chromosomal damage	Chromosomal aberrations	–	10 mg/L	Single dose tested only, for 6, 24, and 96 h Nuclear abnormalities (lobed nuclei, segmented nuclei and kidney-shaped nuclei)	Cavalcante *et al.* (2008)
Fish	*Anguilla anguilla* L. (European eel), peripheral mature erythrocytes	Chromosomal damage	Other (ENA)	+	0.058 mg/L	Time of exposure, 1 and 3 days Chromosomal breakage and/or chromosomal segregational abnormalities after 3 days of exposure, $P < 0.05$	Guilherme *et al.* (2010)
Caiman	*Caiman latirostris* (broad-snouted caiman), erythrocytes	DNA damage	DNA strand breaks, comet assay	+	0.500 mg/egg	Glyphosate, 66.2% In-ovo exposure; blood sampling at the time of hatching $P < 0.05$ in both experiments (50–1000 µg/egg in experiment 1; 500–1750 µg/egg in experiment 2)	Poletta *et al.* (2009)
Caiman	*Caiman latirostris* (broad-snouted caiman), erythrocytes	DNA damage	DNA strand breaks, comet assay	–	19 800 mg/L	Glyphosate, 66.2% Single dose tested only; in-ovo exposure First spraying exposure at the beginning of incubation period, a second exposure on day 35, then incubation until hatching	Poletta *et al.* (2011)
Caiman	*Caiman latirostris* (broad-snouted caiman), erythrocytes	Chromosomal damage	Micronucleus fomation	+	0.500 mg/egg	Glyphosate, 66.2% In-ovo exposure; blood sampling at the time of hatching $P < 0.05$ in both experiments (50–1000 µg/egg in experiment 1; 500–1750 µg/egg in experiment 2)	Poletta *et al.* (2009)

Case 3:16-md-02741-VC Document 654-2 Filed 10/28/17 Page 107 of 138

Case 3:16-md-02741-VC Document 654-2 Filed 10/28/17 Page 108 of 138

Table 4.5 (continued)

Phylogenetic class	Species, strain, tissue	End-point	Test	Results[a]	Dose (LED or HID)	Comments	Reference
Caiman	*Caiman latirostris* (broad-snouted caiman), erythrocytes	Chromosomal damage	Micronucleus fomation	+	19.8 g/L	Glyphosate, 66.2% One dose tested; in-ovo exposure First spraying exposure at the beginning of incubation period, a second exposure on day 35, then incubation until hatching. Micronucleus formation, $P < 0.001$ Damage index, $P < 0.001$	Poletta *et al.* (2011)
Frog tadpole	*Rana catesbeiana* (ouaouaron), blood	DNA damage	DNA strand breaks, comet assay	+	1.687 mg/L, p.o.	Time of exposure, 24 h $P < 0.05$, with 6.75 mg/L; and $P < 0.001$ with 27 mg/L (with 108 mg/L, all died within 24 h)	Clements *et al.* (1997)
Frog	*Eleutherodactylus johnstonei* (Antilles coqui), erythrocytes	DNA damage	DNA strand breaks, comet assay	+	0.5 µg a.e./cm^2	Glyphosate-based formulation, 480 g/L Exposure to an homogenate mist in a 300 cm^2 glass terrarium Time of exposure: 0.5, 1, 2, 4, 8 and 24 h $P < 0.05$	Meza-Joya *et al.* (2013)
Frog	*Euflictis cyanophlyctis* (Indian skittering frog), erythrocytes	Chromosomal damage	Micronucleus formation	+	1 mg a.e./L	Glyphosate isopropylamine salt, 41% Time of exposure: 24, 48, 72, and 96 h $P < 0.001$ at 24, 48, 72 and 96 h	Yadav *et al.* (2013)
Snail	*Biomphalaria alexandrina*, haemolymph	DNA damage	DNA strand breaks, comet assay	+	10 mg/L	Glyphosate, 48% Single dose tested only, for 24 h. The percentage of damaged DNA was 21% vs 4% (control) No statistical analysis	Mohamed (2011)
Oyster	Oysters, spermatozoa	DNA damage	DNA strand breaks, comet assay	–	5 µg/L	Glyphosate, 200 µg equivalent/L Time of exposure, 1 h	Akcha *et al.* (2012)

Table 4.5 (continued)

Phylogenetic class	Species, strain, tissue	End-point	Test	Results[a]	Dose (LED or HID)	Comments	Reference
Clam	*Corbicula fluminea* (Asian clam) haemocytes	DNA damage	DNA strand breaks, comet assay	–	10 mg/L	Time of exposure, 96 h Significant increase when atrazine (2 or 10 mg/L) was added to glyphosate ($P < 0.05$) No increase after exposure to atrazine or glyphosate separately	dos Santos & Martinez (2014)
Mussels	*Utterbackia imbecillis* (Bivalvia: Unionidae) glochidia mussels (larvae)	DNA damage	DNA strand breaks, comet assay	–	5 mg/L	Glyphosate, 18% Doses tested: 2.5 and 5 mg/L for 24 h NOEC, 10.04 mg/L	Conners & Black (2004)
Worm	Earthworm, *Eisenia andrei*, coelomocytes	DNA damage	DNA strand breaks, comet assay	–	240 µg a.e./cm²	Monoammonium salt, 85.4%, a.e. Epidermic exposure during 72 h (on filter paper)	Piola *et al.* (2013)
Worm	Earthworm, *Eisenia andrei*, coelomocytes	DNA damage	DNA strand breaks, comet assay	+	15 µg a.e./cm²	Monoammonium salt, 72%, a.e. Epidermic exposure during 72 h (on filter paper) $P < 0.001$	Piola *et al.* (2013)
Worm	Earthworm, *Pheretima peguana*, coelomocytes	DNA damage	DNA strand breaks, comet assay	–	251.50 µg/cm²	Active ingredient, 36% (w/v) Epidermic exposure 48 h on filter paper; LC_{50}, 251.50 µg/cm²	Muangphra *et al.* (2014)
Worm	Earthworm, *Pheretima peguana*, coelomocytes	Chromosomal damage	Micronucleus formation	+	251.50 µg/cm²	Active ingredient, 36% (w/v) Exposure, 48 h on filter paper; LC_{50}, 251.50 µg/cm² filter paper $P < 0.05$, for total micro-, bi-, and trinuclei frequencies at 0.25 µg/cm²; when analysed separately, micro- and trinuclei frequencies significantly differed from controls only at the LC_{50}	Muangphra *et al.* (2014)

Case 3:16-md-02741-VC Document 654-2 Filed 10/28/17 Page 109 of 138

Table 4.5 (continued)

Phylogenetic class	Species, strain, tissue	End-point	Test	Results[a]	Dose (LED or HID)	Comments	Reference
Insect	*Drosophila melanogaster*	Mutation	Sex-linked recessive lethal mutations	+	1 ppm	Single dose tested only $P < 0.001$	Kale *et al.* (1995)
Plant systems	*Allium cepa* (onion)	Chromosomal damage	Chromosomal aberrations	+	1.44 µg/mL	Glyphosate-based formulation, 480 g/L The doses of formulation were calculated as glyphosate isopropylamine $P < 0.005$	Rank *et al.* (1993)
Plant systems	*Crepis capillaris* (hawksbeard)	Chromosomal damage	Chromosomal aberrations	–	0.5%	The highest dose tested (1%) was toxic	Dimitrov *et al.* (2006)
Plant systems	*Hordeum vulgare* L. cv. Madalin (barley roots)	Chromosomal damage	Chromosomal aberrations	(+)	360 µg/mL (0.1%)	Reported as "significant"	Truta *et al.* (2011)
Plant systems	*Crepis capillaris* (hawksbeard)	Chromosomal damage	Micronucleus formation	–	0.5%	The highest dose tested (1%) was toxic	Dimitrov *et al.* (2006)

[a] +, positive; –, negative; (+) or (–) positive/negative in a study with limited quality

a.e., acid equivalent; AMPA, aminomethyl phosphonic acid; bw, body weight; ENA, erythrocytic nuclear abnormalities; Endo III, endonuclease III; FPG, formamidopyrimidine glycosylase; h, hour; HID, highest ineffective dose; LC_{50}, median lethal dose; LED, lowest effective dose; NOEC, no-observed effect concentration; p.o., oral; SMART, somatic mutation and recombination test

Case 3:16-md-02741-VC Document 654-2 Filed 10/28/17 Page 110 of 138

Table 4.6 Genetic and related effects of glyphosate and glyphosate-based formulations on non-mammalian systems in vitro

Phylogenetic class	Test system (species; strain)	End-point	Test	Results[a]		Concentration (LEC or HIC)	Comments	Reference
				Without metabolic activation	With metabolic activation			
Glyphosate								
Eukaryote Fish	*Oreochromis niloticus* (Nile tilapia), erythrocytes	DNA damage	DNA strand breaks, comet assay	+	NT	7 µM [1.2 µg/mL]	Glyphosate isopropylamine, 96% $P \leq 0.001$; positive dose–response relationship for doses ≥ 7 µM	Alvarez-Moya *et al.* (2014)
Prokaryote (bacteria)	*Scytonema javanicum* (cyanobacteria)	DNA damage	DNA strand breaks, FADU assay	(+)	NT	10 µM [1.7 µg/mL] (in combination with UVB)	Co-exposure to glyphosate (not tested alone; single dose tested only) enhanced UVB-induced increases	Wang *et al.* (2012)
Prokaryote (bacteria)	*Anabaena spherica* (cyanobacteria)	DNA damage	DNA strand breaks, FADU assay	(+)	NT	10 µM [1.7 µg/mL] (in combination with UVB)	Co-exposure to glyphosate (not tested alone; single dose tested only) enhanced UVB-induced increases	Chen *et al.* (2012)
Prokaryote (bacteria)	*Microcystis viridis* (cyanobacteria)	DNA damage	DNA strand breaks, FADU assay	(+)	NT	10 µM [1.7 µg/mL] (in combination with UVB)	Co-exposure to glyphosate (not tested alone; single dose tested only) enhanced UVB-induced increases	Chen *et al.* (2012)
Prokaryote (bacteria)	*Bacillus B. subtilis*	Differential toxicity	Rec assay	–	NT	2000 µg/disk		Li & Long (1988)
Prokaryote (bacteria)	*Salmonella typhimurium* TA1535, TA1537, TA1538, TA98 and TA100	Mutation	Reverse mutation	–	–	5000 µg/plate		Li & Long (1988)
Prokaryote (bacteria)	*Escherichia coli* WP2	Mutation	Reverse mutation	–	–	5000 µg/plate		Li & Long (1988)

Case 3:16-md-02741-VC Document 654-2 Filed 10/28/17 Page 111 of 138

Table 4.6 (continued)

Phylogenetic class	Test system (species; strain)	End-point	Test	Results[a]		Concentration (LEC or HIC)	Comments	Reference
				Without metabolic activation	With metabolic activation			
Acellular systems	Prophage superhelical PM2 DNA	DNA damage	DNA strand breaks	(–)	NT	75 mM [12.7 mg/mL] (in combination with H_2O_2 (100 µM)	Glyphosate inhibited H_2O_2-induced damage of PM2 DNA at concentrations where synergism was observed in cellular DNA damage (data NR)	Lueken *et al.* (2004)
Glyphosate-based formulations								
Prokaryote (bacteria)	*Salmonella typhimurium* TA98	Mutation	Reverse mutation	+	–	360 µg/plate	Glyphosate isopropylammonium salt, 480 g/L	Rank *et al.* (1993)
Prokaryote (bacteria)	*Salmonella typhimirium* TA100	Mutation	Reverse mutation	–	+	720 µg/plate	Glyphosate isopropylammonium salt, 480 g/L	Rank *et al.* (1993)

[a] +, positive; –, negative; (+) or (–) positive/negative in a study with limited quality

FADU, fluorometric analysis of DNA unwinding; HIC, highest ineffective concentration; LEC, lowest effective concentration; NR, not reported; NT, not tested; UVB, ultraviolet B

Case 3:16-md-02741-VC Document 654-2 Filed 10/28/17 Page 112 of 138

Additionally, although all four glyphosate-based formulations dramatically reduced the transcription of ERα and ERβ in ERE-transfected HepG2 cells, glyphosate alone had no significant effect. Glyphosate and all four formulations reduced androgen-receptor transcription in the breast cancer cell line MDA-MB453-kb2, which has a high level of androgen receptor, with the formulations showing greater activity than glyphosate alone.

In a human placental cell line derived from choriocarcinoma (JEG3 cells), 18 hours of exposure to a glyphosate-based formulation (IC_{50} = 0.04%) decreased aromatase activity (Richard *et al.*, 2005). Glyphosate alone was without effect. The concentrations used did not affect cell viability.

Glyphosate, at non-overtly toxic concentrations, decreased aromatase activity in fresh human placental microsomes and transformed human embryonic kidney cells (293) transfected with human aromatase cDNA (Benachour *et al.*, 2007). A glyphosate-based formulation, at non-overtly toxic concentrations, had the same effect. The formulation was more active at equivalent doses than glyphosate alone.

In human androgen receptor and ERα and ERβ reporter gene assays using the Chinese hamster ovary cell line (CHO-K1), glyphosate had neither agonist nor antagonist activity (Kojima *et al.*, 2004, 2010).

(ii) Non-human mammalian experimental systems

In vivo

No data were available to the Working Group.

In vitro

Benachour *et al.* (2007) and Richard *et al.* (2005) reported that glyphosate and a glyphosate-based formulation inhibited aromatase activity in microsomes derived from equine testis. Richard *et al.* (2005) reported an absorbance spectrum consistent with an interaction between a nitrogen atom of glyphosate and the active site of the purified equine aromatase enzyme.

In the mouse MA-10 Leydig cell tumour cell line, a glyphosate-based formulation (glyphosate, 180 mg/L) markedly reduced [$(Bu)_2$] cAMP-stimulated progesterone production (Walsh *et al.*, 2000). The inhibition was dose-dependent, and occurred in the absence of toxicity or parallel reductions in total protein synthesis. In companion studies, the formulation also disrupted steroidogenic acute regulatory protein expression, which is critical for steroid hormone synthesis. Glyphosate alone did not affect steroidogenesis at any dose tested up to 100 µg/L. Forgacs *et al.* (2012) found that glyphosate (300 µM) had no effect on testosterone production in a novel murine Leydig cell line (BLTK1). Glyphosate did not modulate the effect of recombinant human chorionic gonadotropin, which served as the positive control for testosterone production.

(iii) Non-mammalian experimental systems

Gonadal tissue levels of testosterone, 17β-estradiol and total microsomal protein were significantly reduced in adult snails (*Biomphalaria alexandrina*) exposed for 3 weeks to a glyphosate-based formulation (glyphosate, 48%) at the LC_{10} (10% lethal concentration) (Omran & Salama, 2013). These effects persisted after a 2-week recovery period, although the impact on 17β-estradiol was reduced in the recovery animals. The formulation also induced marked degenerative changes in the ovotestis, including absence of almost all the gametogenesis stages. CYP450 1B1, measured by enzyme-linked immunosorbent assay (ELISA), was substantially increased in the treated snails, including after the recovery period.

Glyphosate (0.11 mg/L for 7 days) did not increase plasma vittelogenin levels in juvenile rainbow trout (Xie *et al.*, 2005).

(b) Other pathways

(i) Humans

Studies in exposed humans

No data were available to the Working Group.

Human cells in vitro

Glyphosate did not exhibit agonist activity in an assay for a human pregnane X receptor (PXR) reporter gene in a CHO-K1 cell line (Kojima *et al.*, 2010).

(ii) Non-human mammalian experimental systems

In vivo

In rats, glyphosate (300 mg/kg bw, 5 days per week, for 2 weeks) had no effect on the formation of peroxisomes, or the activity of hepatic carnitine acetyltransferase and catalase, and did not cause hypolipidaemia, suggesting that glyphosate does not have peroxisome proliferator-activated receptor activity (Vainio *et al.*, 1983).

In vitro

Glyphosate was not an agonist for mouse peroxisome proliferator-activated receptors PPARα or PPARγ in reporter gene assays using CV-1 monkey kidney cells in vitro (Kojima *et al.*, 2010). Glyphosate was also not an agonist for the aryl hydrocarbon receptor in mouse hepatoma Hepa1c1c7 cells stably transfected with a reporter plasmid containing copies of dioxin-responsive element (Takeuchi *et al.*, 2008).

(iii) Non-mammalian experimental systems

As a follow-up to experiments in which injection of glyphosate, or incubation with a glyphosate-based formulation (glyphosate, 48%), caused chick and frog (*Xenopus laevis*) cephalic and neural crest terata characteristic of retinoic acid signalling dysfunction, Paganelli *et al.*, (2010) measured retinoic acid activity in tadpoles exposed to a glyphosate-based formulation. Retinoic activity measured by a reporter gene assay was increased by the formulation, and a retinoic acid antagonist blocked the effect. This indicated a possible significant modulation of retinoic acid activity by glyphosate.

4.2.3 Oxidative stress, inflammation, and immunosuppression

(a) Oxidative stress

(i) Humans

Studies in exposed humans

No data were available to the Working Group.

Human cells in vitro

Several studies examined the effects of glyphosate on oxidative stress parameters in the human keratinocyte cell line HaCaT. Gehin *et al.* (2005) found that a glyphosate-based formulation was cytotoxic to HaCaT cells, but that addition of antioxidants reduced cytotoxicity. Elie-Caille *et al.* (2010) showed that incubation of HaCaT cells with glyphosate at 21 mM (the half maximal inhibitory concentration for cytotoxicity, IC_{50}) for 18 hours increased production of hydrogen peroxide (H_2O_2) as shown by dichlorodihydrofluorescein diacetate assay. Similarly, George & Shukla (2013) exposed HaCaT cells to a glyphosate-based formulation (glyphosate, 41%; concentration, up to 0.1 mM) and evaluated oxidative stress using the dichlorodihydrofluorescein diacetate assay. The formulation (0.1 mM) increased maximum oxidant levels by approximately 90% compared with vehicle, an effect similar to that of H_2O_2 (100 mM). Pre-treatment of the cells with the antioxidant *N*-acetylcysteine abrogated generation of oxidants by both the formulation and by H_2O_2. *N*-Acetylcysteine also inhibited cell proliferation induced by the glyphosate-based formulation (0.1 mM). [The Working Group noted the recognized limitations of using dichlorodihydrofluorescein diacetate as a marker of oxidative stress (Bonini *et al.*, 2006; Kalyanaraman *et al.*, 2012),

and that the studies that reported this end-point as the sole evidence for oxidative stress should thus be interpreted with caution.]

Chaufan *et al.* (2014) evaluated the effects of glyphosate, AMPA (the main metabolite of glyphosate), and a glyphosate-based formulation on oxidative stress in HepG2 cells. The formulation, but not glyphosate or AMPA, had adverse effects. Specifically, the formulation increased levels of reactive oxygen species, nitrotyrosine formation, superoxide dismutase activity, and glutathione, but did not have an effect on catalase or glutathione-*S*-transferase activities. Coalova *et al.* (2014) exposed Hep2 cells to a glyphosate-based formulation (glyphosate as isopropylamine salt, 48%) at the LC_{20} (concentration not otherwise specified) and evaluated various parameters of oxidative stress. Exposure to the formulation for 24 hours increased catalase activity and glutathione levels, but did not have an effect on superoxide dismutase or glutathione-*S*-transferase activity.

Using blood samples from non-smoking male donors, Mladinic *et al.* (2009b) examined the effects of in-vitro exposure to glyphosate on oxidative DNA damage in primary lymphocyte cultures and on lipid peroxidation in plasma. Both parameters were significantly elevated at glyphosate concentrations of 580 µg/mL (~3.4 mM), but not at lower concentrations. Kwiatkowska *et al.* (2014) examined the effects of glyphosate, its metabolite AMPA, and *N*-methylglyphosate (among other related compounds) in human erythrocytes isolated from healthy donors. The erythrocytes were exposed at concentrations of 0.01–5 mM for 1, 4, or 24 hours before flow cytometric measurement of the production of reactive oxygen species with dihydrorhodamine 123. Production of reactive oxygen species was increased by glyphosate (≥ 0.25 mM), AMPA (≥ 0.25 mM), and *N*-methylglyphosate (≥ 0.5 mM).

(ii) Non-human mammalian experimental systems

Most of the studies of oxidative stress and glyphosate were conducted in rats and mice, and examined a range of exposure durations, doses, preparations (glyphosate and glyphosate-based formulations), administration routes and tissues. In addition, various end-points were evaluated to determine whether oxidative stress is induced by exposure to glyphosate. Specifically, it was found that glyphosate induces production of free radicals and oxidative stress in mouse and rat tissues through alteration of antioxidant enzyme activity, depletion of glutathione, and increases in lipid peroxidation. Increases in biomarkers of oxidative stress upon exposure to glyphosate in vivo have been observed in blood plasma (Astiz *et al.*, 2009b), liver (Bolognesi *et al.*, 1997; Astiz *et al.*, 2009b), skin (George *et al.*, 2010), kidney (Bolognesi *et al.*, 1997; Astiz *et al.*, 2009b), and brain (Astiz *et al.*, 2009b). Several studies demonstrated similar effects with a glyphosate-based formulation in the liver (Bolognesi *et al.*, 1997; Cavuşoğlu *et al.*, 2011; Jasper *et al.*, 2012), kidney (Bolognesi *et al.*, 1997; Cavuşoğlu *et al.*, 2011) and brain (Cattani *et al.*, 2014), or with a pesticide mixture containing glyphosate in the testes (Astiz *et al.*, 2013). Pre-treatment with antioxidants has been shown to mitigate the induction of oxidative stress by a glyphosate-based formulation (Cavuşoğlu *et al.*, 2011) and by a pesticide mixture containing glyphosate (Astiz *et al.*, 2013).

DNA damage associated with oxidative stress after exposure to glyphosate (e.g. as reported in Bolognesi *et al.*, 1997) is reviewed in Section 4.2.1.

(iii) Non-mammalian experimental systems

Positive associations between exposure to glyphosate and oxidative stress were reported in various tissues in aquatic organisms (reviewed in Slaninova *et al.*, 2009). Glyphosate and various glyphosate-based formulations have been tested in various fish species for effects on a plethora of end-points (e.g. lipid peroxidation, DNA

damage, expression of antioxidant enzymes, levels of glutathione), consistently presenting evidence that glyphosate can cause oxidative stress in fish (Lushchak *et al.*, 2009; Ferreira *et al.*, 2010; Guilherme *et al.*, 2010, 2012a, b, 2014a, b; Modesto & Martinez, 2010a, b; Cattaneo *et al.*, 2011; Glusczak *et al.*, 2011; de Menezes *et al.*, 2011; Ortiz-Ordoñez *et al.*, 2011; Nwani *et al.*, 2013; Marques *et al.*, 2014, 2015; Sinhorin *et al.*, 2014; Uren Webster *et al.*, 2014). Similar effects were observed in bullfrog tadpoles exposed to a glyphosate-based formulation (Costa *et al.*, 2008), and in the Pacific oyster exposed to a pesticide mixture containing glyphosate (Geret *et al.*, 2013).

(b) Inflammation and immunomodulation

(i) Humans

Studies in exposed humans

No data were available to the Working Group.

Human cells in vitro

Nakashima *et al.* (2002) investigated the effects of glyphosate on cytokine production in human peripheral blood mononuclear cells. Glyphosate (1 mM) had a slight inhibitory effect on cell proliferation, and modestly inhibited the production of IFN-gamma and IL-2. The production of TNF-α and IL-1 β was not affected by glyphosate at concentrations that significantly inhibited proliferative activity and T-cell-derived cytokine production.

(ii) Non-human mammalian experimental systems

Kumar *et al.* (2014) studied the pro-inflammatory effects of glyphosate and farm air samples in wildtype C57BL/6 and TLR4$^{-/-}$ mice, evaluating cellular response, humoral response, and lung function. In the bronchoalveolar lavage fluid and lung digests, airway exposure to glyphosate (1 or 100 μg) significantly increased the total cell count, eosinophils, neutrophils, and IgG1 and IgG2a levels. Airway exposure to glyphosate (100 ng, 1 μg, or 100 μg per day for 7 days) also produced substantial pulmonary inflammation, confirmed by histological examination. In addition, glyphosate-rich farm-air samples significantly increased circulating levels of IL-5, IL-10, IL-13 and IL-4 in wildtype and in TLR4$^{-/-}$ mice. Glyphosate was also tested in wildtype mice and significantly increased levels of IL-5, IL-10, IL-13, and IFN-γ (but not IL-4). The glyphosate-induced pro-inflammatory effects were similar to those induced by ovalbumin, and there were no additional or synergistic effects when ovalbumin was co-administered with glyphosate.

Pathological effects of glyphosate on the immune system have been reported in 13-week rat and mouse feeding studies by the NTP (Chan & Mahler, 1992). Relative thymus weight was decreased in male rats exposed for 13 weeks, but increased in male mice. Treatment-related changes in haematological parameters were observed in male rats at 13 weeks and included mild increases in haematocrit [erythrocyte volume fraction] and erythrocytes at 12 500, 25 000, and 50 000 ppm, haemoglobin at 25 000 and 50 000 ppm, and platelets at 50 000 ppm. In female rats, small but significant increases occurred in lymphocyte and platelet counts, leukocytes, mean corpuscular haemoglobin, and mean corpuscular volume at 13 weeks.

Blakley (1997) studied the humoral immune response in female CD-1 mice given drinking-water containing a glyphosate-based formulation at concentrations up to 1.05% for 26 days. The mice were inoculated with sheep erythrocytes to produce a T-lymphocyte, macrophage-dependent antibody response on day 21 of exposure. Antibody production was not affected by the formulation.

(iii) Non-mammalian experimental systems

A positive association between exposure to glyphosate and immunotoxicity in fish has been reported. Kreutz *et al.* (2011) reported alterations

in haematological and immune-system parameters in silver catfish (*Rhamdia quelen*) exposed to sublethal concentrations (10% of the median lethal dose, LC_{50}, at 96 hours) of a glyphosate-based herbicide. Numbers of blood erythrocytes, thrombocytes, lymphocytes, and total leukocytes were significantly reduced after 96 hours of exposure, while the number of immature circulating cells was increased. The phagocytic index, serum bacteria agglutination, and total peroxidase activity were significantly reduced after 24 hours of exposure. Significant decreases in serum bacteria agglutination and lysozyme activity were found after 10 days of exposure. No effect on serum bactericidal and complement natural haemolytic activity was seen after 24 hours or 10 days of exposure to glyphosate.

el-Gendy *et al.* (1998) demonstrated effects of a glyphosate-based formulation (glyphosate, 48%) at 1/1000 of the concentration recommended for field application on humoral and cellular immune response in bolti fish (*Tilapia nilotica*). The mitogenic responses of splenocytes to phytohaemagglutinin, concanavalin A, and lipopolysaccharide in fish exposed to glyphosate for 96 hours were gradually decreased and reached maximum depression after 4 weeks. Glyphosate also produced a concentration-dependent suppression of in-vitro plaque-forming cells in response to sheep erythrocytes.

4.2.4 Cell proliferation and death

(a) Humans

(i) Studies in exposed humans

No data were available to the Working Group.

(ii) Human cells in vitro

Cell proliferation potential was explored in HaCaT keratinocytes exposed to a glyphosate-based formulation (glyphosate, 41%; concentration, up to 0.1 mM) (George & Shukla, 2013). The formulation increased the number of viable cells, as assessed by the MTT assay (based on reduction of the dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) at concentrations up to 0.1 mM, while concentration- and incubation-time-dependent reductions were seen at higher concentrations (up to 1 mM). The formulation (0.01 or 0.1 mM for 72 hours) significantly enhanced cell proliferation (measured by staining for either proliferating cell nuclear antigen or 5-bromo-2′-deoxyuridine); at 0.1 mM, the increases exceeded levels for the positive control, tetradecanoyl-phorbol-13-acetate. The proportion of S-phase cells (assessed using flow cytometry) and the expression of G1/S cell-cycle regulatory proteins (cyclins D1 and E, CDK2, CDK4, and CDK6) increased after exposure to the formulation or the positive control.

Li *et al.* (2013) reported that glyphosate and AMPA inhibited cell growth in eight human cancer cell lines, but not in two immortalized normal prostate cell lines. An ovarian (OVCAR-3) and a prostate (C4–2B) cell line showed the greatest loss in viability, with glyphosate or AMPA at 15–50 mM. Further assays were conducted on AMPA, but not glyphosate, in two prostate cancer cell lines (C4–2B and PC-3), and found cell-cycle arrest (decreased entry of cells into S-phase) and increased apoptosis. [The Working Group noted that the findings from these assays with AMPA are of unclear relevance to the effects of glyphosate.]

Glyphosate (10^{-6} to 1 μM) increased growth by 15–30% relative to controls in hormone-dependent T47D breast cancer cells, but only when endogenous estrogen was minimized in the culture medium (by substitution with 10% dextran-charcoal treated fetal bovine serum). Glyphosate did not affect the growth of hormone-independent MDA-MB231 breast cancer cells cultured in either medium (Thongprakaisang *et al.*, 2013).

Glyphosate (up to 30 μM) did not show cell proliferation potential (5-bromo-2′-deoxyuridine) and did not activate caspase 3 or TP53 in human neuroprogenitor ReN CX cells (Culbreth *et al.*, 2012).

Several studies evaluated the impact of glyphosate or glyphosate-based formulations on apoptotic cell death in the HepG2 human hepatoma cell line. Glyphosate-based formulations induced apoptosis in HepG2 cells, while glyphosate alone was generally without effect or showed effects at considerably higher concentrations (Gasnier *et al.*, 2009, 2010; Mesnage *et al.*, 2013; Chaufan *et al.*, 2014; Coalova *et al.*, 2014). For example, 23.5% of the nuclei of HepG2 cells exposed to a glyphosate-based formulation showed condensed and fragmented chromatin ($P < 0.01$), and caspases 3 and 7 were significantly activated, both effects being indicative of apoptosis (Chaufan *et al.*, 2014). Caspases were unaffected by glyphosate or AMPA alone. Glyphosate and AMPA did not affect cell viability at concentrations up to 1000 mg/L, a concentration that increased rather than decreased cell viability after 48 and 72 hours of incubation. In contrast, cells exposed to glyphosate-based formulation at lower concentrations were not viable. Similarly, Coalova *et al.* (2014) reported that a glyphosate-based formulation (glyphosate, 48%) induced apoptotic cell death in HepG2 cells. Apoptosis was indicated by activation of caspases 3 and 7, and the significant fraction (17.7%) of nuclei with condensed and fragmented chromatin ($P < 0.001$).

In studies with glyphosate and nine different glyphosate-based formulations in three cell lines, glyphosate alone did not increase the activity of adenylate kinase (Mesnage *et al.*, 2013). The activity of caspases 3 and 7 was significantly increased by glyphosate in HepG2 and embryonic kidney HEK293 cells, and elevated (although not significantly) about 1.8 times above control levels in placental choriocarcinoma JEG-3 cells. Two formulations containing an ethoxylated adjuvant induced adenylate kinase activity to a greater extent than caspase activity. All formulations were reported to be more cytotoxic than glyphosate. [In concentration–response curves, glyphosate showed an effect on mitochondrial succinate dehydrogenase activity, a measure of cell viability, that was similar to that shown by one formulation. The calculated 50% lethal concentration in JEG3 cells for mitochondrial succinate dehydrogenase activity was greater for three formulations, although the values appeared inconsistent with the concentration–response curves.]

In HUVEC primary neonate umbilical cord vein cells, and 293 embryonic kidney and JEG3 placental cell lines, Benachour & Séralini (2009) found that glyphosate at relatively high concentrations induced apoptosis, as indicated by induction of caspases 3 and 7, and DNA staining and microscopy. At comparable or lower concentrations, four glyphosate-based formulations all caused primarily necrotic cell death. The umbilical cord HUVEC cells were the most sensitive (by about 100-fold) to the apoptotic effects of glyphosate.

Heu *et al.* (2012) evaluated apoptosis in immortalized human keratinocytes (HaCaT) exposed to glyphosate (5–70 mM). Based on annexin V, propidium iodide and mitochondrial staining, exposures leading to 15% cytotoxicity gave evidence of early apoptosis, while increases in late apoptosis and necrosis were observed at higher levels of cytotoxicity.

(b) Non-human mammalian experimental systems

(i) In vivo

In male Wistar rats, glyphosate (10 mg/kg bw, injected intraperitoneally three times per week for 5 weeks) reduced, but not significantly, the inner mitochondrial membrane integrity of the substantia nigra and cerebral cortex (Astiz *et al.* 2009a). Caspase 3 activity was unaltered in these tissues. Mitochondrial cardiolipin content was significantly reduced, particularly in the substantia nigra, where calpain activity was substantially higher. Glyphosate induced DNA fragmentation in the brain and liver.

(ii) In vitro

In adult Sprague Dawley rat testicular cells exposed in vitro, glyphosate (up to 1%; for 24 or 48 hours) did not provoke cell-membrane alterations (Clair *et al.*, 2012). However, caspase 3 and 7 activity increased with exposure in Sertoli cells alone, and in Sertoli and germ cell mixtures. On the other hand, a glyphosate-based formulation (a 0.1% solution, containing 0.36 g/L of glyphosate) induced membrane alterations and decreased the activity of caspase 3 and 7 in Leydig cells, and in Sertoli and germ cell mixtures. In a separate study, glyphosate increased apoptosis in primary Sertoli cell cultures from mice (Zhao *et al.*, 2013).

Glyphosate (5–40 mM, for 12, 24, 48, or 72 hours) significantly increased cell death in a time- and concentration-dependent manner in differentiated rat pheochromocytoma PC12 (neuronal) cells Gui *et al.* (2012). Apoptotic changes included cell shrinkage, DNA fragmentation, decreased Bcl2 expression, and increased Bax expression. Both autophagy and apoptosis were implicated, as pre-treatment with the pan-caspase inhibitor Z-VAD or the autophagy inhibitor 3-MA inhibited cell loss.

Induction of apoptosis by glyphosate or glyphosate-based formulations was also studied in other cell lines. Glyphosate (10 μM) induced apoptosis in rat heart H9c2 cells, the effect being enhanced when glyphosate was given in combination with the adjuvant TN-20 (5 μM), (Kim *et al.*, 2013). A glyphosate-based formulation induced apoptosis in mouse 3T3-L1 fibroblasts, and inhibited their transformation to adipocytes (Martini *et al.*, 2012). A glyphosate-based formulation (10 mM) did not increase rat hepatoma HTC cell death, but did affect mitochondrial membrane potential (Malatesta *et al.*, 2008).

Glyphosate (up to 30 μM) did not activate caspase 3 or show cell proliferation potential (5-bromo-2′-deoxyuridine) in a mouse neuroprogenitor cell line, but did activate Tp53 at the highest concentration tested (Culbreth *et al.*, 2012).

4.2.5 Other mechanisms

No data on immortalization, epigenetic alterations, altered DNA repair, or genomic instability after exposure to glyphosate were available to the Working Group.

4.3 Data relevant to comparisons across agents and end-points

No data on high-throughput screening or other relevant data were available to the Working Group. Glyphosate was not tested by the Tox21 and ToxCast research programmes of the government of the USA (Kavlock *et al.* 2012; Tice *et al.*, 2013).

4.4 Cancer susceptibility data

No studies that examined genetic, life-stage, or other susceptibility factors with respect to adverse health outcomes that could be associated with exposure to glyphosate were identified by the Working Group.

4.5 Other adverse effects

4.5.1 Humans

In the USA in the past decade, poison-control centres have reported more than 4000 exposures to glyphosate-containing herbicides, of which several hundred were evaluated in a health-care facility, and fatalities were rare (Rumack, 2015). In a pesticide surveillance study carried out by the National Poisons Information Service of the United Kingdom, glyphosate was among the most common pesticide exposure implicated in severe or fatal poisoning cases between 2004 and 2013 (Perry *et al.*, 2014). Deliberate poisonings with glyphosate resulting in toxicity and fatality

have been reported in many countries, including Australia (Stella & Ryan, 2004), Denmark (Mortensen *et al.*, 2000), India (Mahendrakar *et al.*, 2014), Japan (Motojyuku *et al.*, 2008), Republic of Korea (Park *et al.*, 2013), New Zealand (Temple & Smith, 1992), Sri Lanka (Roberts *et al.*, 2010), Taiwan, China (Chen *et al.*, 2009), and Thailand (Sribanditmongkol *et al.*, 2012).

Glyphosate demonstrated no potential for photo-irritation or photo-sensitization in 346 volunteers exposed dermally on normal or abraded skin (Hayes & Laws, 1991). On the other hand, Mariager *et al.* (2013) reported severe burns after prolonged accidental dermal exposure to a glyphosate-based formulation.

4.5.2 Experimental systems

Glyphosate was tested in nine regulatory submissions included in the Toxicity Reference Database (ToxRefDB) and reviewed by the EPA (EPA, 2015). Specifically, study design, treatment group, and treatment-related effect information were captured for four long-term studies and/or carcinogenicity studies, one short-term study, two multigeneration studies of reproductivity, and two studies of developmental toxicity. The NTP also tested glyphosate in a 13-week study in rats and mice (Chan & Mahler, 1992).

In a long-term combined study of toxicity and carcinogenicity in rats given glyphosate at nominal doses of 100, 400, and 1000 mg/kg bw per day, inflammation was observed in the stomach mucosa of females at the intermediate and highest doses (EPA, 1990, 1991b). In males at the highest dose, liver weight, cataracts and lens degeneration in the eyes, and urine specific gravity were increased, while body weight, body-weight gain, and urinary pH were decreased. Pancreatic acinar cell atrophy was observed in males at the highest dose. Pancreatic inflammation was also observed in male rats at the highest dose in a short-term study (nominal doses of 50, 250, and 1000 mg/kg bw per day) (EPA, 1987). In the study by the NTP, cytoplasmic alteration was observed in the parotid and submandibular salivary glands of rats (Chan & Mahler, 1992).

In a study of carcinogenicity in mice given glyphosate at doses of 150, 1500, or 4500 mg/kg bw per day, liver hypertrophy and necrosis were observed in males at the highest dose (EPA, 1983). Other effects in males at the highest dose included increased testes weight, interstitial nephritis, and decreased body weight. In females at the highest dose, ovary weights were increased, proximal tubule epithelial basophilia and hypertrophy was observed, and body weights were decreased. In the study by the NTP, cytoplasmic alteration was observed in the parotid salivary glands in mice (Chan & Mahler, 1992).

Developmental and reproductive toxicity

In a study of developmental toxicity in rats given glyphosate at a dose of 300, 1000, or 3500 mg/kg bw per day, reduced implantation rates and fewer live fetuses were observed in dams at the highest dose (EPA, 1980b). In fetuses at the highest dose, unossified sternebra were observed and fetal weight was reduced.

5. Summary of Data Reported

5.1 Exposure data

Glyphosate is a broad-spectrum herbicide that is effective at killing or suppressing all plant types, including grasses, perennials, and woody plants. The herbicidal activity of glyphosate was discovered in 1970 and since then its use has increased to a point where it is now the most heavily used herbicide in the world, with an annual global production volume in 2012 of more than 700 000 tonnes used in more than 750 different products. Changes in farming practice and the development of genetically modified crops that are resistant to glyphosate have contributed to the increase in use.

There is little information available on occupational or community exposure to glyphosate. Glyphosate can be found in soil, air, surface water and groundwater, as well as in food. It has been detected in air during agricultural herbicide-spraying operations. Glyphosate was detected in urine in two studies of farmers in the USA, in urban populations in Europe, and in a rural population living near areas sprayed for drug eradication in Columbia. However, urinary concentrations were mostly below the limit of detection in several earlier studies of forestry workers who sprayed glyphosate. Exposure of the general population occurs mainly through diet.

5.2 Human carcinogenicity data

In its evaluation of the epidemiological studies reporting on cancer risks associated with exposure to glyphosate, the Working Group identified seven reports from the Agricultural Health Study (AHS) cohort and several reports from case–control studies. The AHS cohort, the pooled analyses of the case–control studies in the midwest USA, and the cross-Canada study were considered key investigations because of their relatively large size. Reports from two or more independent studies were available for non-Hodgkin lymphoma (NHL), multiple myeloma, Hodgkin lymphoma, glioma, and prostate. For the other cancer sites, results from only one study were available for evaluation.

5.2.1 NHL and other haematopoietic cancers

Two large case–control studies of NHL from Canada and the USA, and two case–control studies from Sweden reported statistically significant increased risks of NHL in association with exposure to glyphosate. For the study in Canada, the association was seen among those with more than 2 days/year of exposure, but no adjustment for other pesticides was done. The other three studies reported excesses for NHL associated with exposure to glyphosate, after adjustment for other pesticides (reported odds ratio were 2.1 (95% CI, 1.1–4.0); 1.85 (95% CI, 0.55–6.2); and 1.51 (95% CI, 0.77–2.94). Subtype-specific analyses in a Swedish case–control study indicated positive associations for total NHL, as well as all subtypes, but this association was statistically significant only for the subgroup of lymphocytic lymphoma/chronic lymphocytic leukaemia (OR, 3.35; 95% CI, 1.42–7.89). An elevated risk (OR, 3.1; 95% CI, 0.6–17.1) was also found for B-cell lymphoma in an European study based on few cases. One hospital-based case–control study from France did not find an association between exposure to glyphosate and NHL (OR, 1.0; 95% CI, 0.5–2.2) based on few exposed cases.

A roughly twofold excess of multiple myeloma, a subtype of NHL, was reported in three studies: only among the highest category of glyphosate use (> 2 days/year) in the large Canadian case–control study, in a case–control study from Iowa, USA, and in a French case–control study (all not statistically significant). These three studies did not adjust for the effect of other pesticides. In the AHS, there was no association with NHL (OR, 1.1; 0.7–1.9). For multiple myeloma, relative risk was 1.1 (95% CI, 0.5–2.4) when adjusted for age only; but was 2.6 (95% CI, 0.7–9.4) when adjusted for multiple confounders. No excess in leukaemia was observed in a case–control study in Iowa and Minnesota, USA, or in the AHS.

In summary, case–control studies in the USA, Canada, and Sweden reported increased risks for NHL associated with exposure to glyphosate. The increased risk persisted in the studies that adjusted for exposure to other pesticides. The AHS cohort did not show an excess of NHL. The Working Group noted that there were excesses reported for multiple myeloma in three studies; however, they did not weight this evidence as strongly as that of NHL because of the possibility that chance could not be excluded; none of the

risk estimates were statistically significant nor were they adjusted for other pesticide exposures.

5.2.2. Other cancer sites

No association of glyphosate with cancer of the brain in adults was found in the Upper Midwest Health case–control study. No associations in single case–control studies were found for cancers of the oesophagus and stomach, prostate, and soft-tissue sarcoma. For all other cancer sites (lung, oral cavity, colorectal, pancreas, kidney, bladder, breast, prostate, melanoma) investigated in the large AHS, no association with exposure to glyphosate was found.

5.3 Animal carcinogenicity data

Glyphosate was tested for carcinogenicity in male and female mice by dietary administration in two studies, and in male and female rats by dietary administration in five studies and in drinking-water in one study. A glyphosate-based formulation was also tested in drinking-water in one study in male and female rats, and by skin application in one initiation–promotion study in male mice.

There was a positive trend in the incidence of renal tubule carcinoma and of renal tubule adenoma or carcinoma (combined) in males in one feeding study in CD-1 mice. Renal tubule carcinoma is a rare tumour in this strain of mice. No significant increase in tumour incidence was seen in female mice in this study. In the second feeding study, there was a significant positive trend in the incidence of haemangiosarcoma in male CD-1 mice. No significant increase in tumour incidence was seen in female mice in this study.

For the five feeding studies in rats, two studies in the Sprague-Dawley strain showed a significant increase in the incidence of pancreatic islet cell adenoma in males – one of these two studies also showed a significant positive trend in the incidences of hepatocellular adenoma in males and of thyroid C-cell adenoma in females. Two studies (one in Sprague-Dawley rats, one in Wistar rats) found no significant increase in tumour incidence at any site. One study in Wistar rats was inadequate for the evaluation because of the short duration of exposure.

In the study in Wistar rats given drinking-water containing glyphosate, there was no significant increase in tumour incidence.

A glyphosate-based formulation was found to be a skin-tumour promoter in the initiation–promotion study in male Swiss mice. The study of a glyphosate-based formulation in drinking-water in Sprague-Dawley rats was inadequate for the evaluation because of the small number of animals per group, and the limited information provided on tumour histopathology and incidence in individual animals. These studies of a chemical mixture containing glyphosate were considered inadequate to evaluate the carcinogenicity of glyphosate alone.

5.4. Other relevant data

Direct data on absorption of glyphosate in humans were not available to the Working Group. Glyphosate was detected in the urine of agricultural workers in several studies, and in the blood of poisoning cases, indicative of absorption. Some evidence for absorption through human skin (~2%) was reported in studies in vitro. The minor role of dermal absorption was also shown in a study in non-human primate model in vivo. However, no study examined the rates of absorption in humans. In rodents, several studies showed up to 40% absorption after oral administration of a single or repeated dose.

Glyphosate was measured in human blood. No data on parenchymal tissue distribution for glyphosate in humans were available to the Working Group. In rats given glyphosate by oral administration, concentrations in tissues had the following rank order: kidneys > spleen > fat > liver. Repeated administration had no effect

on the distribution of glyphosate. In a study in rats, the half-life of glyphosate in plasma was estimated to be more than 1 day, indicating that glyphosate is not rapidly eliminated.

In the environment, glyphosate is degraded by soil microbes, primarily to aminomethylphosphonic acid (AMPA) and carbon dioxide. Glyphosate is not efficiently metabolized in humans or other mammals. In rats, small amounts of AMPA were detected in the plasma and in the colon, with the latter being attributed to intestinal microbial metabolism. In humans, small amounts of AMPA are detectable in blood in cases of deliberate glyphosate poisoning. Few studies examined the possible effects of glyphosate-based formulations on metabolizing enzymes, but no firm conclusions could be drawn from these studies.

Studies in rodents showed that systemically absorbed glyphosate is excreted unchanged into the urine, and that the greatest amount is excreted in the faeces, indicating poor absorption. Glyphosate was detected in the urine of humans who were exposed occupationally to glyphosate. AMPA has also been detected in human urine.

Glyphosate is not electrophilic.

A large number of studies examined a wide range of end-points relevant to genotoxicity with glyphosate alone, glyphosate-based formulations, and AMPA.

There is strong evidence that glyphosate causes genotoxicity. The evidence base includes studies that gave largely positive results in human cells in vitro, in mammalian model systems in vivo and in vitro, and studies in other non-mammalian organisms. In-vivo studies in mammals gave generally positive results in the liver, with mixed results for the kidney and bone marrow. The end-points that have been evaluated in these studies comprise biomarkers of DNA adducts and various types of chromosomal damage. Tests in bacterial assays gave consistently negative results.

The evidence for genotoxicity caused by glyphosate-based formulations is strong. There were three studies of genotoxicity end-points in community residents exposed to glyphosate-based formulations, two of which reported positive associations. One of these studies examined chromosomal damage (micronucleus formation) in circulating blood cells before and after aerial spraying with glyphosate-based formulations and found a significant increase in micronucleus formation after exposure in three out of four different geographical areas. Additional evidence came from studies that gave largely positive results in human cells in vitro, in mammalian model systems in vivo and in vitro, and studies in other non-mammalian organisms. The end-points that were evaluated in these studies comprised biomarkers of DNA adducts and various types of chromosomal damage. The pattern of tissue specificity of genotoxicity end-points observed with glyphosate-based formulations is similar to that observed with glyphosate alone. Tests in bacterial assays gave generally negative results.

For AMPA, the evidence for genotoxicity is moderate. While the number of studies that examined the effects of AMPA was not large, all of the studies gave positive results. Specifically, genotoxicity was reported in a study in humans in vitro, a study in mammals in vivo, a study in mammals in vitro, and one study in eels in vivo.

Strong evidence exists that glyphosate, AMPA, and glyphosate-based formulations can induce oxidative stress. Evidence came from studies in many rodent tissues in vivo, and human cells in vitro. In some of these studies, the mechanism was challenged by co-administration of antioxidants and observed amelioration of the effects. Similar findings have been reported in fish and other aquatic species. Various end-points (e.g. lipid peroxidation markers, oxidative DNA adducts, dysregulation of antioxidant enzymes) have been evaluated in numerous studies. This

increased the confidence of the Working Group in the overall database.

There is weak evidence that glyphosate or glyphosate-based formulations induce receptor-mediated effects. In multiple experiments, glyphosate-based formulations affected aromatase activity; glyphosate was active in a few of these studies. Some activity in other nuclear receptor-mediated pathways has been observed for glyphosate or glyphosate-based formulations. In one series of experiments, glyphosate was not found to be a ligand to several receptors and related proteins (aryl hydrocarbon receptor, peroxisome proliferator-activated receptors, pregnane X receptor).

There is weak evidence that glyphosate may affect cell proliferation or death. Several studies in human and rodent cell lines have reported cytotoxicity and cell death, the latter attributed to the apoptosis pathway. Studies that examined the effects of glyphosate alone or a glyphosate-based formulation found that glyphosate alone had no effect, or a weaker effect than the formulation.

There is weak evidence that glyphosate may affect the immune system, both the humoral and cellular response, upon long-term treatment in rodents. Several studies in fish, with glyphosate or its formulations, also reported immunosuppressive effects.

With regard to the other key characteristics of human carcinogens (IARC, 2014), the Working Group considered that the data were too few for an evaluation to be made.

Severe or fatal human poisoning cases have been documented worldwide. In rodents, organ and systemic toxicity from exposures to glyphosate are demonstrated by liver-weight effects and necrosis in animals at high doses. Additionally, effects on the pancreas, testes, kidney and ovaries, as well as reduced implantations and unossified sternebra were seen at similar doses.

No data on cancer-related susceptibility after exposure to glyphosate were available to the Working Group.

Overall, the mechanistic data provide strong evidence for genotoxicity and oxidative stress. There is evidence that these effects can operate in humans.

6. Evaluation

6.1 Cancer in humans

There is *limited evidence* in humans for the carcinogenicity of glyphosate. A positive association has been observed for non-Hodgkin lymphoma.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of glyphosate.

6.3 Overall evaluation

Glyphosate is *probably carcinogenic to humans (Group 2A).*

6.4 Rationale

In making this overall evaluation, the Working Group noted that the mechanistic and other relevant data support the classification of glyphosate in Group 2A.

In addition to limited evidence for the carcinogenicity of glyphosate in humans and sufficient evidence for the carcinogenicity of glyphosate in experimental animals, there is strong evidence that glyphosate can operate through two key characteristics of known human carcinogens, and that these can be operative in humans. Specifically:

- There is strong evidence that exposure to glyphosate or glyphosate-based formulations is genotoxic based on studies in humans in vitro and studies in experimental animals.

One study in several communities in individuals exposed to glyphosate-based formulations also found chromosomal damage in blood cells; in this study, markers of chromosomal damage (micronucleus formation) were significantly greater after exposure than before exposure in the same individuals.

- There is strong evidence that glyphosate, glyphosate-based formulations, and aminomethylphosphonic acid can act to induce oxidative stress based on studies in experimental animals, and in studies in humans in vitro. This mechanism has been challenged experimentally by administering antioxidants, which abrogated the effects of glyphosate on oxidative stress. Studies in aquatic species provide additional evidence for glyphosate-induced oxidative stress.

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