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REVIEW ARTICLE

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A review of the carcinogenic potential of glyphosate by four independent expert panels and comparison to the IARC assessment

Gary M. Williams[a], Marilyn Aardema[b], John Acquavella[c], Sir Colin Berry[d], David Brusick[e], Michele M. Burns[f], Joao Lauro Viana de Camargo[g], David Garabrant[h], Helmut A. Greim[i], Larry D. Kier[j], David J. Kirkland[k], Gary Marsh[l], Keith R. Solomon[m], Tom Sorahan[n], Ashley Roberts[o] and Douglas L. Weed[p]

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ABSTRACT

The International Agency for Research on Cancer (IARC) published a monograph in 2015 concluding that glyphosate is "probably carcinogenic to humans" (Group 2A) based on limited evidence in humans and sufficient evidence in experimental animals. It was also concluded that there was strong evidence of genotoxicity and oxidative stress. Four Expert Panels have been convened for the purpose of conducting a detailed critique of the evidence in light of IARC's assessment and to review all relevant information pertaining to glyphosate exposure, animal carcinogenicity, genotoxicity, and epidemiologic studies. Two of the Panels (animal bioassay and genetic toxicology) also provided a critique of the IARC position with respect to conclusions made in these areas. The incidences of neoplasms in the animal bioassays were found not to be associated with glyphosate exposure on the basis that they lacked statistical strength, were inconsistent across studies, lacked dose-response relationships, were not associated with preneoplasia, and/or were not plausible from a mechanistic perspective. The overall weight of evidence from the genetic toxicology data supports a conclusion that glyphosate (including GBFs and AMPA) does not pose a genotoxic hazard and therefore, should not be considered support for the classification of glyphosate as a genotoxic carcinogen. The assessment of the epidemiological data found that the data do not support a causal relationship between glyphosate exposure and non-Hodgkin's lymphoma while the data were judged to be too sparse to assess a potential relationship between glyphosate exposure and multiple myeloma. As a result, following the review of the totality of the evidence, the Panels concluded that the data do not support IARC's conclusion that glyphosate is a "probable human carcinogen" and, consistent with previous regulatory assessments, further concluded that glyphosate is unlikely to pose a carcinogenic risk to humans.

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Introduction

Background on glyphosate

Glyphosate, or N-(phosphonomethyl)glycine (CAS# 1071-83-6), is a widely used broad-spectrum, nonselective post-emergent herbicide that has been in use since 1974. Glyphosate effectively suppresses the growth of many species of trees, grasses, and weeds. Glyphosate works by interfering with the synthesis of the aromatic amino acids phenylalanine, tyrosine, and tryptophan, through the inhibition of the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS). Inhibition of the synthesis of these amino acids stops growth of plants such as weeds. Importantly, EPSPS is not present in mammals, which obtain their essential aromatic amino acids from the diet.

A wide variety of new uses have been developed for glyphosate in agricultural, industrial, and home & garden applications. Glyphosate accounts for approximately 25% of the global herbicide market (http://www.glyphosate.eu). Glyphosate is currently marketed under numerous trade names by more than 50 companies in several hundreds of crop protection products around the world. More than 160 countries have approved uses of glyphosate-based herbicide products (http://www.monsanto.com). To further enhance the effectiveness of glyphosate in agriculture, a number of genetically modified crop varieties have been developed which are tolerant to glyphosate (i.e. allows for application after emergence of the crops). In addition, given its effectiveness and broad-spectrum activity, glyphosate is also used worldwide for forestry, rights of way, landscape, and household control of weeds.

Glyphosate is a relatively simple molecule which consists of the amino acid glycine and a phosphonomethyl moiety (Figure 1). As such, glyphosate has no structural alerts for chromosomal damage, genotoxicity, mutagenicity, or carcinogenicity when analyzed by DEREK (Deductive Estimation of Risk from Existing Knowledge) (Kier & Kirkland 2013). It is a polar molecule that is incompletely (15–36%) absorbed orally, undergoes very little biotransformation, and is rapidly excreted unmetabolized (Williams et al. 2000). A molecule with these characteristics would be expected to exhibit, if any, only a low order of toxicity. The results from toxicity studies and regulatory risk assessments have been consistent with that expectation (JMPR 1987, 2006; US EPA 1993; WHO 1994; Williams et al. 2000; European Commission 2002; EFSA 2015).

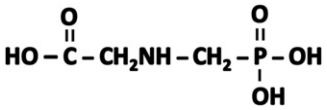

Figure 1. Structure of glyphosate.

Previous assessments of the carcinogenicity of glyphosate

The safety, including the potential carcinogenicity, of glyphosate has been reviewed by scientists and regulatory authorities worldwide, including the US Environmental Protection Agency (US EPA), the European Commission, and the Canadian Pest Management Regulatory Agency (Health and Welfare Canada 1991; US EPA 1993, 2013; WHO 1994; Williams et al. 2000; European Commission 2002; Kier & Kirkland 2013; EFSA 2015; Health Canada 2015; JMPR 2016). The conclusion of all these reviews is that proper use of glyphosate and glyphosate-based formulations (GBFs) does not pose a genotoxic or carcinogenic hazard/risk to humans.

The first assessment of glyphosate's carcinogenic potential was undertaken by the US EPA in 1985. This review was done by a US EPA panel that then was called the Toxicology Branch Ad Hoc Committee, which comprised members of the Toxicology Branch of the Hazard Evaluation Division. At that time, two chronic animal bioassays were available: a combined chronic toxicity/carcinogenicity study in Sprague-Dawley rats and a carcinogenicity study in CD-1 mice. The Agency concluded that the data did not demonstrate a carcinogenic response in rats. However, the US EPA also concluded that the dose levels used in that study were inadequate for assessing glyphosate's carcinogenic potential in this species. The US EPA concluded that there was limited evidence of an increased incidence of renal tubule adenomas in male mice at the high-dose level (4841 mg/kg/day), a dose that greatly exceeds the limit dose level (1000 mg/kg/day) for carcinogenicity testing with pesticides (OECD 2009). Based on this information, the Agency initially classified glyphosate as a Group C (Possibly Carcinogenic to Humans: Agents with limited animal evidence and little or no human data) carcinogen (see US EPA 1991a).

The kidney slides from the mouse study were subsequently reexamined by a consulting pathologist (Dr. Marvin Kuschner M.D., Dean, School of Medicine, State University of New York at Stony Brook), and three other scientists (Dr. Robert A. Squire, Robert A. Squire Associates Inc., Ruxton Maryland; Dr. Klaus L. Stemmer M.D., Kettering Laboratory, University of Cincinnati Medical Center; Dr. Robert E. Olson, M.D., Ph.D., Professor of Medicine and Pharmacological Sciences, State University of New York at Stony Brook) also reviewed the slides and/or the chronic toxicity data. All these scientists concluded that there was no relationship to treatment (US EPA, 1986a). In addition, a Pathology Working Group (PWG), consisting of 5 pathologists (Dr. RM Sauer, Dr. MR Anver, Dr. JD Strandberg, Dr. JM Ward, and Dr. DG Goodman), was also assembled and they issued the following conclusion: "This PWG firmly believes and unanimously concurs with the original pathologist and reviewing pathologist that the incidences of renal tubular cell neoplasms in this study are not compound related" (US EPA 1986a).

All available information was presented to an US EPA FIFRA Science Advisory Panel (SAP) in February 1986. The SAP determined that the carcinogenic potential of glyphosate could not be determined from the existing data and proposed that a chronic rat and/or mouse study be conducted

in order to clarify these unresolved questions; the panel also proposed that glyphosate be categorized as Group D or having "inadequate animal evidence of oncogenicity" (US EPA 1986b).

After considering the SAP's conclusions and recommendations, the US EPA requested that a new 2-year rat oncogenicity study be conducted. In 1991, after the new rat study was completed, the US EPA re-convened its Carcinogenicity Peer Review Committee to review the results of this study as well as all of the relevant scientific data on glyphosate (US EPA 1991a). The Committee concluded that glyphosate should be classified in Group E (evidence of non-carcinogenicity) based upon the lack of a carcinogenic response in two animal species. Subsequent reevaluations by US EPA (1993, 2012, 2013) have re-affirmed the Agency's earlier conclusion.

After Monsanto had marketed glyphosate-based herbicide products for a number of years, other companies entered the glyphosate market; as a result, some of them generated substantial, or even complete, additional toxicology databases. The first additional databases that became available were generated by Cheminova and Syngenta in the mid- to late 1990s timeframe. Additional data packages were subsequently generated by other companies (e.g. Arysta, Excel, Feinchemie, Nufarm) and became available in the mid- and late 2000s timeframe.

In addition to new studies conducted to meet regulatory guidelines and support various re-registration processes globally, new epidemiology and genotoxicity studies (testing glyphosate and glyphosate-based herbicide formulations) began to appear in the scientific literature in the late 1990s and early 2000s. One of the first epidemiological investigations of interest involving glyphosate published in the scientific literature was that of Hardell and Eriksson (1999), and other epidemiology studies were periodically published after 2000 up until the present. Genetic toxicology studies of glyphosate and GBFs began to appear in the literature in increasing numbers throughout the 1990s and were reviewed by Williams et al. (2000). The occurrence of such studies has increased during the 2001–2015 timeframe: approximately 125 such genotoxicity studies were reviewed by Kier and Kirkland (2013), and an additional 40 genotoxicity biomonitoring studies of GBFs were reviewed by Kier (2015).

As glyphosate underwent reregistration processes by major national regulatory authorities and additional reviews by other health agencies after 2000, these evaluations included more and more of the new toxicology, genotoxicity, and epidemiology information generated after the initial Monsanto animal bioassay studies. For example, a 2004 Joint Meeting of the FAO Panel of Experts on Pesticide Residues (JMPR) in Food and the Environment and the WHO Core Assessment Group concluded that there was an absence of carcinogenic potential in animals and a lack of genotoxicity in standard tests; thus, "the Meeting concluded that glyphosate is unlikely to pose a carcinogenic risk to humans" (JMPR 2006). The Australian Pesticides and Veterinary Medicines Authority (APVMA) evaluated the active ingredient and concluded that the evidence shows that glyphosate is not genotoxic or carcinogenic (APVMA 2013). The US EPA conducted a comprehensive Human Health Risk Assessment in 2012 (US EPA 2012). The Agency noted that "no evidence of carcinogenicity was found in mice or rats," and US EPA concluded that "glyphosate does not pose a cancer risk to humans" (US EPA 2013). Health Canada's Pesticide Management Regulatory Agency (PMRA) completed a comprehensive review of glyphosate as part of the reregistration process in that country. PMRA concluded that "the overall weight of evidence indicates that glyphosate is unlikely to pose a human cancer risk" (Health Canada 2015). The complete genotoxicity, carcinogenicity, and human epidemiology databases were evaluated by the German Federal Institute for Risk Assessment (BfR) for the European Commission on the Annex 1 renewal of glyphosate. The BfR concluded that glyphosate is unlikely to pose a carcinogenic risk to humans (Markard 2014). This conclusion was supported by the peer review evaluation conducted by the European Food Safety Authority (EFSA) both before and after a mandate from the European Commission to consider the findings from IARC regarding glyphosate's carcinogenic potential (EFSA 2015). Most recently, JMPR (2016) reviewed the data and concluded that: "glyphosate is unlikely to pose a carcinogenic risk to humans from exposure through the diet."

IARC assessment of the carcinogenicity of glyphosate

The International Agency for Research on Cancer (IARC) in 2015 undertook an evaluation of the oncogenic potential of glyphosate as part of its Monograph Programme. Glyphosate, along with four other pesticides (the insecticides diazinon, malathion, parathion, and tetrachlorvinphos), was considered by an IARC Working Group, which met in March 2015 at IARC in Lyon, France. A brief summary of IARC's conclusions was initially published in The Lancet Oncology on 20 March 2015 (Guyton et al. 2015), and the full IARC Monograph (Volume 112) was published online on 29 July 2015 (IARC 2015). IARC concluded that glyphosate is "probably carcinogenic to humans (Group 2A)" based on *limited evidence* in humans and *sufficient evidence* in experimental animals; it was also concluded that there was strong evidence of genotoxicity and oxidative stress (IARC 2015).

Expert Panel critique of the IARC assessment and review of relevant data

Since the IARC conclusions were found to be in such stark contrast to those from all other assessments of carcinogenic potential, it was decided that a thorough review should be conducted by scientists in the area of cancer risk assessment, critiquing IARC's processes where appropriate. Toward that end, Intertek Scientific & Regulatory Consultancy (Intertek, Mississauga, Ontario, Canada) was commissioned by the Monsanto Company to assemble panels of scientific experts in the four areas considered by IARC: exposure; epidemiology; cancer in experimental animals; mechanistic and other relevant data (focused on genotoxicity and oxidative stress).

Fifteen scientific experts were selected on the basis of their expertise and standing within the international scientific community (i.e. publication history, participation in scientific

Table 1. Composition of the four Expert Panels.

Expert panel group*	Name of participating scientist	Affiliation of scientist
Human exposures	Keith R. Solomon	Centre for Toxicology, University of Guelph, Guelph, ON Canada
Carcinogenicity bioassays	Gary M. Williams	Professor of Pathology, New York Medical College, Valhalla, NY
	Sir Colin Berry	Emeritus Professor of Pathology, Queen Mary, University of London, London, UK
	Michele M. Burns	Boston Children's Hospital, Boston, MA, USA
	Joao Lauro Viana de Camargo	Professor of Pathology, Botucatu Medical School, São Paulo State Univ, UNESP, SP, Brazil
	Helmut A. Greim	Emeritus Professor of Toxicology and Environmental Hygiene, Technical University of Munich, Germany
Genotoxicity	David Brusick	Toxicology Consultant, Bumpass, VA, USA
	Marilyn Aardema	Marilyn Aardema Consulting, LLC, Fairfield, OH, USA
	Larry D. Kier	Private Consultant, Buena Vista, CO USA
	David J. Kirkland	Kirkland Consulting, Tadcaster, UK
	Gary M. Williams	Professor of Pathology, New York Medical College, Valhalla, NY
Epidemiology	John Acquavella	Professor, Department of Clinical Epidemiology, Aarhus University, Denmark
	David Garabrant	EpidStat Institute; Emeritus Professor of Occupational Medicine and Epidemiology, University of Michigan
	Gary Marsh	Professor of Biostatistics, Director and Founder, Center for Occupational Biostatistics & Epidemiology, University of Pittsburgh, Graduate School of Public Health, Pittsburgh, PA, USA
	Tom Sorahan	Professor of Occupational Epidemiology, University of Birmingham, Birmingham, UK
	Douglas L. Weed	DLW Consulting Services, LLC; Adjunct Professor, University of New Mexico School of Medicine, Albuquerque, NM, USA

*Ashley Roberts of Intertek Scientific & Regulatory Consultancy served as facilitator for each of the four panels.

and regulatory committees, and familiarity with regulatory authorities) and recruited by Intertek to participate on these Expert Panels. Panelists were recruited and assigned to one of the four areas considered by IARC (noted above) based on their areas of expertise; two panelists participated in two areas. A sixteenth scientific expert from Intertek participated on the Expert Panels and served as the overall organizer and facilitator for the panel meetings. A listing of the experts, their affiliations, and the specific "Panel" on which they served is presented in Table 1.

Prior to the meeting, all key studies/publications cited by IARC were made available to the panelists for their review; panelists were told to request any additional information they felt was necessary for them to conduct a thorough evaluation. The epidemiology panel conducted its own independent literature search. The scientists were asked to closely examine the studies/data that IARC used to come to their conclusions; panelists were also advised to examine any additional information needed to come to an overall conclusion in their respective areas.

Based on the scope of the information to be evaluated, it was decided that the panels would meet over a 2-day period to discuss all relevant information and make appropriate conclusions regarding the carcinogenic potential of glyphosate. As needed, the expert scientists held pre-meeting phone conferences and communicated *via* email to establish and plan how they would prepare for and conduct their review at the Expert Panels review meeting. Since the amount, nature, and quality of the data used by IARC varied considerably across the four areas, the evaluation approaches used by the expert panelists in their specialist areas varied somewhat as well. The Expert Panels Meeting was held on 27–28 August 2015 at Intertek in Mississauga, Canada. On the first day of the meeting, the discussions focused on the exposure and human epidemiology data. The second day of the meeting began with a summation of epidemiology and exposure discussions/conclusions and then focused on the animal bioassay and genotoxicity/oxidative stress data. After the Expert Panels met, the reports for the four individual areas were developed by designated scientists; the content of these reports was finalized through additional phone conferences and email communications as necessary with the other panel members. As indicated previously, due to the large amount of data and information evaluated by the individual panels and the subsequent length of the individual reports, it was decided to prepare four separate specialist manuscripts covering the methodologies applied and their respective outcomes and conclusions. This report presents a summary of the deliberations, and conclusions reached, by the Expert Panels in the four areas of research. Prior to publishing the Expert Panels findings, they were presented at the Society for Risk Analysis Annual Meeting at Arlington, Virginia on 7 December 2015.

As a preface to the remainder of the document, the process by which IARC identifies and reviews data must be compared with that employed by the Expert Panel(s). IARC only reviews data included in: "reports that have been published or accepted for publication in the openly available scientific literature" or "data from governmental reports that are publicly available" (IARC 2006). In addition, IARC reviews and assesses these data in the context of hazard (i.e. inherent carcinogenic potential) not risk (i.e. the likelihood of carcinogenic effects at exposure levels humans may encounter). As a result, the conclusion of IARC is often solely associated with hazard. In contrast to IARC, toxicology, mechanism, and exposure Expert Panels evaluated all of the available scientific data, including the results of a number of unpublished reports, some of which have been submitted to and reviewed by regulatory authorities. These reports document GLP- and OECD/FDA Redbook guideline compliant studies, conducted to assess the genotoxic and carcinogenic potential of glyphosate. In essence, these studies provide the highest quality of documentation and verification; hence, a balanced assessment requires the inclusion of such studies in the review process. The third panel (epidemiology) took an approach consistent with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines for systematic reviews (Moher et al. 2009), standard approaches to critically evaluating epidemiologic studies (Aschengrau & Seage

2003a,b; Sanderson et al. 2007) and well-recognized interpretative methods – e.g. the criteria-based methods of causal inference (Hill 1965, 1971) – sometimes referred to as "weight of evidence" (WoE) methods (Weed 2005). In addition to the identification of hazard potential, the Expert Panels assessed exposure data to provide a perspective from which to comment on potential risk. In the absence of carcinogenic hazard, however, no risk is present regardless of exposure. The conclusions reached by the Expert Panels and IARC clearly differ. However, in the opinion of the Expert Panel(s) this is not due to differences in process (hazard versus risk assessment), but rather the result of the exclusion from the IARC review process of key data (animal bioassay and genotoxicity) or differences in the interpretation of the data that was assessed particularly in regard to the animal bioassay results. Given these differences, even without the data IARC did not include, there is no support for IARC's conclusion that glyphosate is "probably carcinogenic to humans." This critique is presented and discussed in the context of the Expert Panels' assessment of the totality of the data.

Exposures to glyphosate

Unpublished reports of studies on exposure to glyphosate in applicators were provided by Monsanto Company which covered uses in agriculture and forestry (see Solomon 2016 for additional details and bibliography). Other data on exposures were obtained from the open literature as a result of searches in PubMed®, references in reviews, and Google Scholar®. These papers and reports were grouped into sources of exposures and the data analyzed as described below.

Only one paper reported concentrations of glyphosate in air. In a study conducted in Iowa, Mississippi, and Indiana in 2007 and 2008, concentrations of glyphosate and its major environmental degradate, aminomethylphosphonic acid (AMPA), were measured in air and precipitation (Chang et al. 2011). For estimation of human exposure, it was assumed that there was 100% absorption of glyphosate from the air into the body of a 70 kg human breathing 8 m^3 air (half a day for an adult) (US EPA 2009). Also, surface water measurements of glyphosate as part of the National Water-Quality Assessment (NAWQA) program (USGS 2015) since 2002 were downloaded from the NAWQA data warehouse and then sorted by concentration. All values measured across the US between 2002 and 2014 were pooled for the analysis. Where concentrations were less than the level of detection (0.02 μg glyphosate acid equivalents (a.e.)/L), these values were substituted with a dummy value of "zero." Although chlorine and ozone are highly effective in removing glyphosate and AMPA during purification of drinking water (Jönsson et al. 2013), it was assumed that treatment did not remove any glyphosate. The estimated concentrations are thus a worst-case.

Studies documenting exposures through food and to "bystanders" (persons who are located within or directly adjacent to areas where pesticides are applied but who are not actively involved in the process) were reviewed and data extracted (Acquavella et al. 2004; Curwin et al. 2007; Mesnage et al. 2012; Hoppe 2013; Honeycutt & Rowlands 2014; Niemann et al. 2015). For those measurements, publications that provided actual systemic dose calculations were used rather than estimates calculated from default exposure factors (e.g. body weight (bw), water consumption, breathing rate, etc.). Where dietary exposures were calculated the urinary concentration was used to calculate the systemic dose on the assumption of 2 L of urine per day and a 60 kg person (Niemann et al. 2015). In 2013, the JMPR reviewed dietary exposures to glyphosate (glyphosate, N-acetyl glyphosate, AMPA, and N-acetyl AMPA) and calculated the international estimated daily intakes (IEDI) of glyphosate for 13 regional food diets (JMPR 2014). These IEDIs were based on estimated mean residues from supervised trials under normal or good agricultural practice. The US EPA has calculated exposures to glyphosate using the Dietary Exposure Evaluation Model (DEEM, ver 7.81), based on tolerance levels for all commodities and modeled estimates of exposures from food and drinking water for the overall US population (US EPA 2012). For studies using dosimetry, the normalization to systemic dose was conducted using the following assumptions: 70 kg adult, 2.1 m^2 surface area for a 70 kg male (US EPA 2009), 10% penetration through clothing if not actually measured, 1% dermal penetration. The estimated systemic doses were ranked from smallest to largest and a cumulative frequency distribution derived. These values were plotted on a log-probability scale. The median (50th centile) and 90th centile values were calculated from the raw data using the Excel function $<=$percentile$>$.

Where an applicator makes a single application, the systemic dose of glyphosate can be estimated from the total amount of glyphosate excreted in the urine over the 4 or 5 days following and including the day of application (Acquavella et al. 2004). If applications are conducted every day, the amount excreted each day provides a time-weighted average for daily exposures. Because glyphosate is applied infrequently in normal agricultural practice, the assumption of a single initial exposure is considered appropriate for risk assessment purposes.

Exposures via air

Based on the above assumptions, inhaling glyphosate in air at the maximum measured concentration would result in an exposure of 1.04×10^{-6} mg/kg body mass (bm)/day. This is about five orders of magnitude less than the systemic ADI proposed by EFSA (2015).

Exposures via water

The concentrations of glyphosate measured in US surface waters ranged from 0.02 to 73 μg/L. The 90th centile value was 0.79 μg/L (see Solomon (2016) for details of the calculations), more than four orders of magnitude less than the EFSA ADI.

Exposures from food and in bystanders

Estimates of glyphosate exposures to bystanders and the general public have been reported by various investigators

(Curwin et al. 2007; Mesnage et al. 2012; Hoppe 2013; Honeycutt & Rowlands 2014; Krüger et al. 2014; Markard 2014). In these studies, the range for estimates of systemic doses was 0.000022–0.00063 mg/kg/day. These values are all less than the ADI suggested by EFSA.

Exposure of applicators

The 90th centile in the dosimetry studies was 0.021 mg/kg/day; about five-times less than the systemic EFSA ADI. The range of values for the systemic doses determined by biomonitoring was smaller than for the passive dosimeters. The 90th centile was 0.0014 mg/kg b.m./day; about 70-times less than the systemic EFSA ADI.

In summary, there is a robust dataset on glyphosate exposures to humans. Even when using worst-case assumptions, systemic exposures to applicators, bystanders, and the general public are very small. Based on current RfDs and ADIs and measured exposures, there is an extremely large margin of safety from exposure to glyphosate *via* normal uses.

Epidemiological data

The epidemiology Expert Panel conducted a systematic review of the published glyphosate literature for the two cancers that were the focus of IARC's epidemiology review: non-Hodgkin's lymphoma (NHL) and multiple myeloma (MM) (see Acquavella et al. (2016) for additional details). Initially, an exhaustive search of the medical literature was performed to identify all epidemiological studies that examined the relationships between reported use of glyphosate and NHL or MM. This resulted in seven unique studies for NHL and four studies for MM after removal of duplicates and focusing on the most recent findings for study populations that were the subject of more than one publication. The relevant studies are listed in Table 2. Each study was then reviewed individually according to key validity considerations specified *a priori* and the results for NHL and MM were separately and systematically evaluated according to widely used criteria for judging causal associations from epidemiologic studies (Hill 1965).

Data abstracted from each study included: first author, year of publication, outcome (NHL, MM), study design, study size, statistical methods, results (measure of relative risk [RR] with accompanying 95% confidence interval [95% CI]), exposure-response findings, and variables controlled in the analyses. Each study was evaluated for key features that relate to study validity, most importantly: recall bias, proxy respondents, selection bias, adequate statistical control for confounding factors, and evaluation of dose response (Table 3).

Of the seven NHL studies, only one study – the Agricultural Health Study (AHS) cohort study (de Roos et al. 2005) – was devoid of major concerns about recall bias and selection bias by virtue of the design (prospective versus retrospective), was controlled comprehensively for confounding factors, and extensively considered RR by frequency and duration of glyphosate use. This study of more than 50 000 licensed pesticide farmers and applicators collected information about pesticide use before follow-up for health outcomes, had only first-hand respondents reporting about pesticide use (viz. no proxy respondents), had minimal potential for selection bias, and included statistical analyses that controlled confounding factors by myriad personal characteristics and non-glyphosate occupational exposures. In addition, de Roos et al. (2005) were the only investigators who conducted exposure-response analyses while controlling extensively for confounding exposures. In contrast, the NHL case–control studies had major validity concerns including the strong potential for recall bias, selection bias (either appreciably lesser participation for controls than cases or selecting controls that clearly did not reflect the population that gave rise to the cases [e.g. hospitals controls from rheumatology and orthopedic departments]), proxy respondents, and uncontrolled confounding factors in the statistical analyses. Indeed, in many of the case–control studies virtually every pesticide exposure studied was associated with increased risk for NHL (or MM) – a clear indication of widespread systematic bias.

With these considerations in mind, for NHL, the results of the de Roos et al. (2005) cohort study were considered the only reliable epidemiologic findings. As de Roos et al. (2005)

Table 2. Relevant studies for glyphosate review: non-Hodgkin's lymphoma (NHL) and multiple myeloma (MM).

First author (year)	Study location(s)	Study design	More recent analysis	Outcome
Cantor et al. (1992)	Iowa + Minnesota	Case–control	de Roos et al. (2003)	NHL
Nordstrom et al. (1998)	Sweden	Case–control	Hardell et al. (2002)	HCL
Hardell and Eriksson (1999)	Sweden	Case–Control	Hardell et al. (2002)	NHL excluding HCL
McDuffie et al. (2001)	Canada	Case–control	n/a	NHL
Hardell et al. (2002)	Sweden	Case–control (pooled)	n/a	NHL + HCL
de Roos et al. (2003)	Nebraska,Iowa/Minnesota,Kansas	Case–control (pooled)	n/a	NHL
de Roos et al. (2005)	Iowa, North Carolina	Cohort	n/a	NHL, MM
Eriksson et al. (2008)	Sweden	Case–control	n/a	NHL
Orsi et al. (2009)	France	Case–control	n/a	NHL, MM
Hohenadel et al. (2011)	Canada	Case–control	Extension of McDuffie et al. (2001)	NHL
Cocco et al. (2013)	Czech, France, Germany, Ireland, Italy, Spain	Case–control	n/a	B-cell lymphoma
Brown et al. (1993)	Iowa	Case–control	n/a	MM
Landgren et al. (2009)	Iowa North Carolina Minnesota	Prevalence, Case–control	n/a	MGUS
Pahwa et al. (2012)	Canada	Case–control	Kachuri et al. (2013)	MM
Kachuri et al. (2013)	Canada	Case–control	n/a	MM
Sorahan (2015)	Iowa, North Carolina	Cohort	Reanalysis of de Roos et al. (2005)	MM

n/a: not available.

Table 3. Key validity considerations in glyphosate epidemiological studies.

First author (year)	Study design	Outcome	Recall bias	Selection bias	Proxy respondents	Adequate control for confounding	Exposure-response and trend test
de Roos et al. (2005)	Cohort	NHL, MM	No	Unlikely	No	Yes	Yes, yes
McDuffie et al. (2001)	Case–control	NHL	Likely	Likely	21% cases 15% controls	No	Yes, no trend test
Hardell et al. (2002)	Case–control	NHL, HCL	Likely	Unlikely	43% NHL cases and controls, 0% for HCL	No	No
de Roos et al. (2003)	Case–control	NHL	Likely	Likely	31% for cases; 40% for controls	Yes	No
Eriksson et al. (2008)	Case–control	NHL	Likely	Unlikely	No	No	Yes, no trend test
Orsi et al. (2009)	Case–control	NHL, MM	Likely	Likely	No	No	No
Cocco et al. (2013)	Case–control	NHL	Likely	Likely	No	No	No
Brown et al. (1993)	Case–control	MM	Likely	Unlikely	42% for cases; 30% for controls	No	No
Kachuri et al. (2013)	Case–control	MM	Likely	Likely	Excluded in analysis	No	Yes, no trend test

NHL: non-Hodgkin's lymphoma; MM: multiple myeloma.
Whether recall bias, exposure misclassification, or selection bias was classified as likely or unlikely was based on a consensus after an in person discussion of each study by the authors.

concluded "… the available data provided evidence of no association between glyphosate exposure and NHL incidence." Results from this study were the basis for the Panel's conclusion of no epidemiologic support for a causal relationship between reported glyphosate use and NHL.

The glyphosate literature for MM is appreciably sparser than the literature for NHL, both in terms of the number of available studies (one cohort and three case–control studies) and the number of cases in those studies with reported glyphosate use. The three case–control studies had important validity concerns, as noted for the NHL case–control studies, and were unable to adjust analyses comprehensively for confounding factors due to the very small number of exposed cases. The AHS cohort study (de Roos et al. 2005 and re-analyzed by Sorahan 2015) found that glyphosate users had about the same rate of MM as non-users adjusting for confounding factors, but had too few exposed cases to conduct informative exposure response analyses.

In summary, the epidemiology Expert Panel concluded that the glyphosate epidemiologic literature does not indicate a causal relationship between glyphosate exposure and NHL. For MM, the evidence was considered too sparse to judge a relationship between MM and reported glyphosate use. The panel's conclusion for NHL differed from that of the IARC working group primarily because the null findings from the AHS (cohort) study were the only epidemiologic findings considered likely to be valid.

Cancer bioassays

The carcinogenicity Expert Panel reviewed all listed cancer bioassays reviewed by Greim et al. (2015) and IARC (2015). The recommended method for evaluating the results of an extensive database of toxicology and carcinogenicity bioassays, as exist for glyphosate, involves the application of a WoE approach (US EPA 1986c; ECHA 2010). Methods for evaluating the results of an extensive database of toxicology and carcinogenicity bioassays, as exist for glyphosate, have evolved from the application of WoE approaches (US EPA, 2005; Suter and Cormier, 2011) to approaches built on the systematic and rigorous methods of systematic evidence-based reviews (James et al. 2015). These approaches recommend that all reliable information be evaluated. Transparent descriptions of studies to be included and excluded are a key component of this approach. In any review, if certain studies are judged to be unreliable and thus not included, the reasons for this should be provided. The carcinogenicity Expert Panel reviewed the incidences of the tumors in the various studies with respect to dose-response, rate of occurrence relative to known spontaneous rates in control animals, and on the basis of biological plausibility. Additional details of the Expert Panel's considerations and conclusions are presented in Williams et al. (2016).

In contrast to the results of past reviews (see Table 4), IARC (2015) concluded that there is *sufficient evidence in experimental animals* for the carcinogenicity of glyphosate, based on the following:

a. A significant positive trend in the incidence ($p = .037$) of renal tubule carcinomas and of adenomas and carcinomas ($p = .034$) in male CD-1 mice of one study only. This is a rare tumor type.
b. In a second feeding study in the same strain of mice, a significant positive trend in the incidence ($p < .001$) of hemangiosarcomas occurred in males.
c. In two dietary studies in SD rats, a significant positive trend ($p < .05$) in the incidence of pancreatic islet cell adenomas occurred in males.
d. In a dietary study in SD rats, a significant positive trend ($p = .016$) in the incidence of hepatocellular adenomas occurred in males.
e. In a dietary study in SD rats, a significant positive trend ($p = .031$) in the incidence of thyroid C-cell adenomas occurred in females.

Kidney tubular – cell neoplasia in mice

In regard to the rare renal tubular tumors in male CD-1 mice, the Expert Panel noted that the conclusions of the IARC were based on only one 2-year oral mouse carcinogenicity study, (Monsanto 1983) excluding two additional 18-month oral studies in CD-1 mice (Arysta Life Sciences 1997; Nufarm 2009)

Table 4. Regulatory agency reviews of three studies evaluated by IARC.

	Regulatory authorities	Conclusions of review – tumors related to treatment?		
		Mouse study (Monsanto 1983)	Rat study (Stout & Ruecker 1990)	Mouse study (Cheminova 1993)
2015	WHO/IARC	Yes	Yes	Yes
2016	WHO/JMPR	*	No	*
2016	US EPA Registration Review**	–	–	–
2016	Japan Food Safety Commission ADI Review**	No	No	–
2015	EU Annex I Renewal (BFR)**	No	No	No
2015	Canada PMRA Registration Review**	No	No	No
2013	Australia	No	No	No
2012	US EPA Human Health RA	No	No	–
2005	WHO/Water Sanitation Health	No	No	
2004	WHO/JMPR	–	No	No
2002	EU Annex I	No	No	No
1999	Japan Food Safety Commission	No	No	–
1994	WHO/IPCS	No	No	–
1993	US EPA RED	No	No	–
1991	Canada PMRA	No	No	–
1991	US EPA Cancer Classification	No	No	–
1987	WHO/JMPR	No	–	–

*The meeting could not exclude the possibility that glyphosate is carcinogenic in mice at very high doses.
**Evaluation not completed.

and one 18-month oral study in Swiss Albino mice (Feinchemie Schwebda 2001). All of the studies were considered by authoritative bodies to have met the guidelines for a carcinogenicity bioassay in mice (US EPA 1990; ICH 1997).

In the study conducted by Monsanto (1983) considered by IARC (2015) to show evidence of renal tubular neoplasia associated with glyphosate dosing, male (M) and female (F) CD-1 mice received 0 (M0/F0 mg/kg/day, control), 1000 (157/190, LD), 5000 (814/955, MD), or 30,000 (4841/5874, HD) ppm in the diet. The incidence by dose of renal neoplasms in male mice was as follows: 1/49, 0/49, 1/50, and 3/50. The important non-neoplastic renal findings of hyperplasia were as follows: 3/49, 0/49, 4/50, and 2/50, indicating lack of a dose-response, with the highest incidence in the mid-dose (MD) group, followed by the control group, and the high-dose (HD) group. The low-dose (LD) group had no renal findings. Females had neither neoplasia nor hyperplasia. Absence of hyperplasia indicates that all renal proliferative and neoplastic lesions, which occurred in all experimental groups (including controls) occurred *de novo*, i.e. were spontaneous or background lesions and were not compound related.

Factors to assess whether an association between exposure and an effect (two variables) is causal include strength, consistency, and specificity of the association, the temporal (latency) and dose-response relationships present, plausibility of effect, and coherence of the available data. When applied to the study by Monsanto (1983), several conclusions were drawn, as follows:

1. The association was not strong because the incidence of rare renal neoplasms was not statistically significant in any exposed group when compared to the control group.
2. The association is not consistent, since four out of five mouse studies did not find similar renal neoplasms at similar doses.
3. The association is not specific, since females of this pivotal study, which were exposed to higher levels of glyphosate, did not develop renal neoplasms. Also, there were no renal findings (hyperplasia, neoplasia) in the LD group, whereas the control group had four.
4. The time required between exposure and effect, i.e. the latency time, was not reduced; all tumors were observed only at termination. Also, no mouse with neoplasia had also hyperplasia.
5. The biological gradient of association or the dose-response curve was absent, since the females and the males in the LD group had no neoplasms, whereas there was one in the control group.
6. A plausible explanation for the association was absent, since the mode of action for induction of these renal neoplasms was not established.
7. Coherence of the association was also absent, as female mice and male and female rats did not display kidney effects. Also in the other four mouse carcinogenicity studies (three of which were not considered in the IARC monograph), the mice did not develop similar neoplastic renal lesions.
8. The association does not demonstrate a dose-response pattern (see #5, 6), and furthermore the "in-study" females had neither neoplasms nor any of the other renal lesions, although they were exposed to higher levels of glyphosate.

Consequently, under the conditions of this assessment, the renal neoplastic effects are not plausibly associated with glyphosate exposure. This conclusion is in agreement with that of JMPR (1987, 2006), US EPA (1993), and EFSA (2015).

Hemangiosarcomas in mice

With respect to the common liver hemangiosarcoma in male mice, in the CD-1 mouse study reported by Cheminova (1993) there were no statistically significant increases in the incidence of any tumors when compared with the in-study and historical (for both sexes 2–12%) control groups and no dose response was apparent (Williams et al. 2016). IARC,

based on their own statistical analysis, indicated/reported that there was an increase in the incidence of hemangiosarcoma in males [$p < .001$, Cochran–Armitage trend test] based on the incidence of the HD group (Table 5). In addition, IARC (2015) did not comment on the lack of hemangiosarcomas in females which have received higher doses of glyphosate, and also of renal tumors in this mouse study.

It is clear that the association between glyphosate treatment and hemangiosarcoma in mice is weak since pairwise comparisons are not significant, there is no consistency (some mouse studies show no tumors of this type at all at comparable doses), and a dose response effect is not seen (some HD groups have a lower incidence than lower doses). In addition, the recorded incidences are within the historical control range.

Given the foregoing analysis, the Expert Panel concludes that overall the evidence does not support the conclusion that glyphosate exposure results in increased incidence of hemangiosarcoma in mice.

Table 5. Tumor incidence/number of animals examined (mg/kg bw/day)*.

	Males				Females			
	0	100	300	1000	0	100	300	1000
Hemangiosarcoma	0/50	0/50	0/50	4/50 (8%)	0/50	2/50 (4%)	0/50	1/50 (2%)

*Taken from Greim et al. (2015).

Table 6. Sprague-Dawley male rats, hepatocellular tumor rates+, and Cochran–Armitage trend and Fisher's exact tests results (p values).

	Dose (ppm)			
Tumors	0	2000	8000	20 000
Carcinomas	3/34	2/45	1/49	2/48†
(%)	(7)	(4)	(2)	(4)
p	.324	.489	.269	.458
Adenomas	2/44	2/45	3/49	7/48‡
(%)	(5)	(4)	(6)	(15)
p	.016*	.683	.551	.101
Adenoma + carcinoma	5/44	4/45	4/49	9/48
(%)	(11)	(9)	(8)	(19)
p	.073	.486	.431	.245
Hyperplasia only	0/44	0/45	1/49¶	0/48
(%)	(0)	(0)	(2)	(0)
p	.462	1.000	.527	1.000

Source: US EPA (1991a,b).
*Number of tumor-bearing animals/number of animals examined, excluding those that died or were sacrificed before week 55.
†First carcinoma observed at week 85 at 20 000 ppm.
‡First adenoma observed at week 88 at 20 000 ppm.
¶First hyperplasia observed at week 89 at 8000 ppm.
Significance of trend denoted at Control. Significance of pair-wise comparison with control denoted at dose level. If then $p < .05$.

Pancreatic tumors in rats

In two of the seven carcinogenicity studies in rats that were evaluated by IARC, tumors of islet cells of the pancreas were diagnosed in both males and females. Both studies were made available to IARC by the US EPA (1991a,b,c).

In the first study Sprague-Dawley rats received doses of 0, 30 (3), 100 (10), and 300 (31 mg/kg bw/day) ppm in the diet for 26 months. No pancreatic islet carcinomas were observed. Adenomas were found having a positive trend ($p < .05$) in the study. The level of significance for an increase in common tumors in the trend test should be $p < .005$. The tumor incidences for controls, low, mid, and high doses respectively were: males – 0/50, 5/49 (10%), 2/50 (4%), 2/50 (4%), and females – 2/50 (4%), 1/50 (2%), 1/50 (2%) 0/50. This incidence demonstrates no dose-response pattern, and an absence of pre-neoplastic effects. In addition, in the first study in males, the adenomas did not progress to carcinomas.

In the second study Sprague-Dawley rats received 0, 2000, 8000, and 20,000 ppm glyphosate (96.5% purity) in the diet, fed *ad libitum* for 24 months. In males, the following pancreatic islet cell tumor incidences were observed in the controls and three dose groups (low to high): adenoma: 1/58 (2%), 8/57 (14%), 5/60 (8%), 7/59 (12%); carcinoma: 1/58 (2), 0/57, 0/60, 0/59. Corresponding incidence values in females were: 5/60 (8%), 1/60 (2%), 4/60 (7%), 0/59, and 0/60, 0/60, 0/60, 0/59. The historical control rates for pancreatic islet cell tumors at the testing laboratory were in the range 1.8–8.5%. The Panel disagrees with the conclusion of IARC that there is a significant positive trend ($p < .05$) in the incidence of pancreatic adenomas in males, since here again the level of significance should be $p < .005$ (US FDA, 2001; Williams et al. 2014). Moreover, there was no progression of adenomas to carcinomas.

Four additional studies in rats, described by Greim et al. (2015) not evaluated by IARC, similarly did not show pancreatic islet cell tumors. Based on this information the Expert Panel concludes that there is no evidence that glyphosate induces islet cell tumors in the pancreas.

Liver tumors in rats

Hepatocellular neoplasms are common for the SD rat (about 5% in males and 3% in female controls) (Williams et al. 2014).

The IARC evaluation indicated that there was "…a significant ($p = .016$) positive trend in the incidences of hepatocellular adenoma in males…" (IARC 2015). This opinion was based on its interpretation of the Stout and Ruecker (1990) study as presented by the US EPA's Peer Review of Glyphosate (US EPA 1991a,b) (see Table 6). The Stout and Ruecker (1990) study has been reviewed twice by the US EPA (1991a,b). The final interpretation of the US EPA Review committee was: "Despite the slight dose-related increase in hepatocellular adenomas in males, this increase was not significant in the pair-wise comparison with controls and was within the historical control range. Furthermore, there was no progression from adenoma to carcinoma and incidences of hyperplasia were not compound-related. Therefore, the slight increased occurrence of hepatocellular adenomas in males is not considered compound-related" (US EPA 1991b). The US EPA ultimately concluded that glyphosate should be classified as a Group E (evidence of non-carcinogenicity for humans) chemical (US EPA 1991a,b).

There are other aspects of the Stout and Ruecker (1990) data that support the conclusion that glyphosate did not exert an oncogenic effect on the liver of SD rats. For example, chemically induced rat hepatocellular carcinogenesis is a multiple stage process characterized by progressive

functional, morphological, and molecular changes that indicate or precede the full establishment of neoplasia, such as enzyme induction, hepatocyte hypertrophy, degeneration and necrosis, hepatocyte proliferation, altered hepatocellular foci, etc. (Williams 1980; Bannasch et al. 2003; Maronpot et al. 2010). Identification and analyses of these liver changes – that span from adaptive to irreversible toxic effects – can help support characterization of key events along the carcinogenesis process and inform the mode of action of the tested chemical (Williams & Iatropoulos 2002; Holsapple et al. 2006; Carmichael et al. 2011). These changes were not apparent in this study.

In the last 30 years, the systemic carcinogenic potential of glyphosate has been assessed in at least eight studies in Sprague-Dawley or Wistar rats, which were not all included within the IARC monograph (Greim et al. 2015); a ninth could not be evaluated because of a high mortality and the low doses used (Chruscielska et al. 2000). Considered jointly, the animals were exposed through the diet to 24 different doses distributed across a wide range (3.0–1290 mg/kg bw/day). In exposed males, the incidences of hepatocellular adenomas across the doses showed no dose-response relationship and varied within the same range as the controls. Similar rates were also seen for hepatocellular carcinomas. These observations confirm that glyphosate is not carcinogenic to the rat liver.

Thyroid tumors in rats

C-cell tumors of the thyroid are a common tumor in the SD rat (Williams et al. 2014).

The incidence of thyroid C-cell adenoma was reported in the Monograph (IARC 2015), to have a significant positive trend ($p = .031$) in females. IARC based their opinion, again, on their interpretation of the Stout and Ruecker's (1990) study and the US EPA's Second Peer Review of Glyphosate (US EPA 1991a). In the Stout and Ruecker's study (1990), no statistically significant difference (group comparison) was reported in the incidence of thyroid C-cell neoplasms, as shown in Table 7. Additionally, the US EPA (1991a) concluded that "the C-cell adenomas in males and females are not considered compound-related." Although the C-cell adenomas were slightly numerically greater in male and female MD and HD groups, there was no dose-related progression to carcinoma and no significant dose-related increase in severity of grade or incidence of hyperplasia in either sex. However, IARC concluded that "there was a statistically significant positive trend in the incidence of thyroid, C-cell adenomas in females" ($p = .031$ but, because this is a common tumor type, the trend significance value should be $p < .005$ (US FDA 2001; Williams et al. 2014)). Thus, this tumor is not significant.

Table 7. Tumor incidence/number of animals examined (mg/kg bw/day)*.

	Males				Females			
	0	89	362	940	0	113	457	1183
Thyroid C-cell adenoma	2/60	4/58	8/58	7/60	2/60	2/60	6/60	6/60
Thyroid C-cell carcinoma	0/60	2/58	0/58	1/58	0/60	0/60	1/60	0/60

*Stout and Ruecker (1990) (all deaths reported).

Therefore, in one of the two evaluated studies, the significant trend in the incidence of thyroid C-cell adenomas in female rats did not materialize, and there was no progression to carcinomas. The adenomas were within the historical ranges.

Genetic toxicity and oxidative stress data

The genetic toxicology Expert Panel (Brusick et al. 2016) considered published studies reviewed in the IARC monograph and additional published studies identified by literature searches or from review articles, not considered by IARC. These included both genetic toxicology studies and studies of oxidative stress. A large number of core genetic toxicology regulatory studies were also considered by the Expert Panel for which information was available from review publication supplements. These regulatory studies were not considered in the IARC monograph, but the Expert Panel concluded that sufficient test-related information was available to justify including these studies. In addition, some unpublished regulatory studies not reviewed previously were included in the Expert Panel evaluation.

The universally recommended method for evaluating the databases of the type associated with glyphosate (including GBFs and AMPA), involves the application of a WoE approach as discussed recently for genetic toxicology testing (US FDA 2006; Dearfield et al. 2011). One of the most important requirements of a WoE approach is that individual test methods should be assigned a weight that is consistent with their contribution to the overall evidence, and different types of evidence or evidence categories must be weighted before they are combined into a WoE.

The weight of a category of evidence used in the Expert Panel evaluation is based on four considerations: (i) different categories of evidence (i.e. assay types) have different weights, (ii) the aggregate strength (robustness of protocols and reproducibility) and quality of evidence in the category also influence the weight (Klimisch et al. 1997), (iii) the number of items of evidence within a category influences the weight, and (iv) tests with greater potential to extrapolate results to humans carry greater weight. In general, human and *in vivo* mammalian systems have the highest test system weight, with a lower weight applied to *in vitro* mammalian cell systems and *in vivo* non-mammalian systems and lowest weight to *in vitro* non-mammalian systems (with the exception of the well-validated bacterial reverse mutation-[Ames] test using mammalian metabolic activation). Typically, the results of *in vivo* assays supersede the results of *in vitro* assays (EFSA 2011).

In contrast to the standard WoE approach used by the Expert Panel, IARC's process for evaluating/weighting the genotoxicity data for glyphosate, GBF and AMPA was not defined. IARC's process may be inferred by how the data were summarized and described, and indicate a number of differences from current standard procedures for WoE. For instance, it appears that IARC considered *in vitro* studies in human cells as carrying more weight than rodent *in vivo* studies as evidenced by the order of discussion topics in Section 4.2.1, and the inclusion of a separate table for human *in vitro* studies. Further, the IARC conclusion of

strong evidence of genotoxicity was stated as based on "studies in humans *in vitro* and studies in experimental animals." In contrast, the Expert Panel evaluation considered *in vitro* studies using cells of human origin to be weighted as equivalent to any other *in vitro* mammalian cell assay using the same endpoint. IARC also gave weight to publications in which glyphosate or GBFs have been tested for genotoxicity in a variety of nonstandard non-mammalian species (fish, insects). The Expert Panel did not consider data from these non-mammalian systems and nonstandard tests with glyphosate, GBF and AMPA to have weight in the overall genotoxicity evaluation, especially given the large number of standard core studies assessing the more relevant gene mutation and chromosomal effects categories available in mammalian systems. In addition, nonstandard tests lack internationally accepted guidelines for design and conduct, databases that document acceptable negative control data or positive control responses are absent, and validation with respect to concordance with rodent or human carcinogenicity has yet to be completed. OECD guidelines specifically state that use of any nonstandard tests require justification along with stringent validation including establishing adequate historical negative and positive control databases (OECD 2014).

In addition, the IARC review seemed to apply significant weight to "indicator" tests such as DNA damage (comet assay) or sister chromatid exchange (SCE) studies. These tests are identified as indicators because the measured endpoint is reversible and does not always lead to mutation, a key event in cancer development. As stated by OECD (2015), when evaluating potential genotoxicants, more weight should be given to the measurement of permanent DNA changes than to DNA damage events that are reversible. Therefore, the Expert Panel also considered that the data from these "indicator" tests with glyphosate, GBFs and AMPA should not have significant weight in the overall genotoxicity evaluation, especially given the large number of standard core studies in the more relevant gene mutation and chromosomal effects categories available in mammalian systems.

IARC did not consider the chemical structure of glyphosate in its mechanistic section. Many guidelines recommend that the presence of structural alerts be considered in evaluation of or testing for genotoxicity (Cimino 2006; Eastmond et al. 2009; EFSA 2011; ICH 2011). As reported in Kier and Kirkland (2013), analysis of the glyphosate structure by DEREK software identified no structural alerts for chromosomal damage, genotoxicity, mutagenicity, or carcinogenicity. The lack of structural alerts in the glyphosate molecular structure suggests lack of genotoxicity and that genotoxic effects observed might be secondary to toxicity or resulting from mechanisms other than DNA reactivity.

Genetic toxicology tests relied upon by most regulatory bodies to support decisions regarding safety focus on a set of core endpoints that are known to be involved either in direct activation of genes responsible for neoplastic initiation in somatic cells or alteration of the genetic information in germ cells (EFSA 2011; ICH 2011; Kirkland et al. 2011). Therefore, the endpoints given the greatest weight in Table 8 consist of gene mutation and chromosomal aberrations.

An evaluation of the studies in Table 8 according to their relative contributions to a WoE produced the following results:

- Test methods identified as providing low contribution to the WoE (low weight) produced the highest frequency of positive responses, regardless of whether the responses were taken from the results of IARC-evaluated studies alone (8 of 9) or from all studies combined (8 of 11).
- The highest frequencies of positive responses were reported for test endpoints and systems considered most likely to yield false or misleading positive results due to their susceptibility to secondary effects. This relationship was constant regardless of whether the results were taken from IARC-evaluated studies alone or all studies combined.
- The numbers of studies providing strong evidence of relevant genotoxicity (high weight) were in the minority for both the IARC and the Expert Panel's evaluations, with 6 out of 15 studies identified as high weight being positive for the IARC evaluation, and only 8 out of 92 studies identified as high weight being positive for all studies combined.

In summary, the WoE from *in vitro* and *in vivo* mammalian tests for genotoxicity indicates that:

- Glyphosate does not induce gene mutations *in vitro*. There are no *in vitro* mammalian cell gene mutation data for GBFs or AMPA, and no gene mutation data *in vivo*.
- Glyphosate, GBFs, and AMPA are not clastogenic *in vitro*. Glyphosate is also not clastogenic *in vivo*. Some positive *in vivo* chromosomal aberration studies with GBFs are all subject to concerns regarding their reliability or biological relevance.
- There is limited evidence that glyphosate induces micronuclei (MN) *in vitro*. Although this could be a reflection of increased statistical power in the *in vitro* MN studies, the absence of clastogenic effects suggests the possibility of threshold-mediated aneugenic effects. However, there is strong evidence that glyphosate does not induce MN *in vivo*.
- Limited studies and potential technical problems do not present convincing evidence that GBFs or AMPA induce MN *in vitro*. The overwhelming majority of *in vivo* MN studies on GBFs gave negative results, but conflicting and limited data do not allow a conclusion on *in vivo* induction of MN by AMPA.
- There is evidence that glyphosate and GBFs can induce DNA strand breaks *in vitro*, but these are likely to be secondary to toxicity since they did not lead to chromosome breaks. There is limited evidence of transient DNA strand breakage for glyphosate and GBFs *in vivo*, but for glyphosate at least these are not associated with DNA adducts. These results are assigned a lower weight than results from other more relevant endpoints, which were more abundant.
- There is evidence that glyphosate and AMPA do not induce unscheduled DNA synthesis (UDS) in cultured hepatocytes.

Table 8. Summary of the Panel's evaluation of human, non-human mammalian and selected microbial genotoxicity studies from IARC section 4.2.1 and other published sources.

Source	Test category	Endpoint	Weight	Glyphosate (Pos/Neg)	GBFs (Pos/Neg)	AMPA (Pos/Neg)	Total (Pos/Neg)
Kier and Kirkland (2013) and other published studies not Included in IARC	Bacterial reverse mutation	Gene mutation	High	0/19	0/20	0/1	0/40
	Mammalian *in vitro*	Gene mutation	Moderate	0/2	ND	ND	0/2
		Chromosomal aberrations	Moderate	1/5	1/0	ND	2/5
		Micronucleus	Moderate	2/0	1/0	ND	3/0
		UDS	Low	0/1	ND	0/1	0/2
		SCE	None	ND	1/0	ND	1/0
	Mammalian *in vivo*	Chromosomal aberrations	High	0/1	2/0	ND	2/1
		Micronucleus	High	0/13	0/17	0/1	0/31
		SCE	None	ND	1/0	ND	1/0
IARC monograph 112	Bacterial reverse mutation	Gene mutation	High	0/1	0/0	ND	0/1
	Mammalian *in vitro*	Gene mutation	Moderate	0/1	ND	ND	0/1
		Chromosomal aberrations	Moderate	1/2	ND	1/0	2/2
		Micronucleus	Moderate	2/0	ND	1/0	3/0
		Comet/DNA breaks	Low	5/0	2/0	1/0	8/0
		UDS	Low	0/1	ND	ND	0/1
		SCE	None	3/0	2/0	ND	5/0
	Mammalian *in vivo*	Chromosomal aberrations	High	0/1	1/1	ND	1/2
		Micronucleus	High	2/1	2/3	1/0	5/4
		Comet/DNA breaks	Moderate	1/0	1/0	ND	2/0
		Dominant lethal	High	0/1	ND	ND	0/1
	Human *in vivo*	Chromosomal aberrations	High	ND	0/1	ND	0/1
		Micronucleus	High	ND	0/3	ND	0/3
	High weight *Combined totals (IARC results only)*			2/37 (2/4)	5/45 (3/5)	½ (1/0)	8/84 (6/9)
	Moderate weight *Combined totals (IARC results only)*			7/10 (4/3)	3/0 (1/0)	2/0 (2/0)	12/10 (7/3)
	Low weight *Combined totals (IARC results only)*			5/2 (5/1)	2/0 (2/0)	1/1 (1/0)	8/3 (8/1)

ND: no data.
All responses based on study critiques and conclusions of Expert Panel members.
Non-mammalian responses from IARC Monograph in this table did not include 4 positive studies measuring DNA strand breaks in bacteria and 1 negative Rec assay in bacteria from Monograph Table 4.6.

Table 9. Summary of studies presented in Kier and Kirkland (2013) and of other publicly available studies not included in the IARC review.

Test category	Endpoint	Glyphosate (Pos/Neg)	GBFs (Pos/Neg)	AMPA (Pos/Neg)	Total (Pos/Neg)
Non-mammalian (bacterial reverse mutation)	Gene mutation	0/19	0/20	0/1	0/40
Mammalian *in vitro*	Gene mutation	0/2	ND	ND	0/2
	Chromosomal aberrations	1/5	1/0	ND	2/5
	Micronucleus	2/0*	1/0	ND	3/0
	UDS	0/1	ND	0/1	0/2
	SCE	ND	1/0	ND	1/0
Mammalian *in vivo*	Chromosomal aberrations	0/1	2/0*	ND	2/1
	Micronucleus	0/13*	0/17	0/1	0/31
	SCE	ND	1/0	ND	1/0
Total		3/41	6/37	0/3	9/81

*Inconclusive studies not included in count. ND: not done.

- Reports of the induction of SCE *in vitro* by glyphosate and GBFs, and one positive report of SCE induction *in vivo* by a GBF, do not contribute to the overall evaluation of genotoxic potential since the mechanism of induction and biological relevance of SCE are unclear.

Although IARC policies prohibited the inclusion of additional data from unpublished studies or governmental reports, it was the Expert Panel's conclusion that the regulatory genetic toxicology studies published in reviews such as Kier and Kirkland (2013) (Table 9) should be included in a WoE assessment. The rationale supporting the inclusion of these additional studies is that the supplementary tables presented in the Kier and Kirkland (2013) paper, contain sufficient detail supporting the reliability of the studies. Failure to evaluate and consider the large number of results included in the publication by Kier and Kirkland (2013), as well as other publicly available studies not reviewed by IARC, results in an inaccurate assessment of glyphosate, GBFs and AMPA's genotoxic hazard/risk potential.

Based on the results of the WoE critique detailed above and the wealth of regulatory studies reviewed by Kier and Kirkland (2013) and Williams et al. (2000), the Panel concluded that the available data do not support IARC's conclusion that there is strong evidence for genotoxicity across the glyphosate or GBFs database. In fact, the Panel's WoE assessment provides strong support for a *lack* of genotoxicity, particularly in the relevant mechanism

Table 10. Comparison of test response profiles from glyphosate, GBFs, and AMPA to the profile characteristics of confirmed genotoxic carcinogens.

Characteristic	Carcinogens with a proven genotoxic mode of action	Glyphosate, GBFs, and AMPA study data
Profile of test responses in genetic assays	Positive effects across multiple key predictive endpoints (i.e. gene mutation, chromosome aberrations, aneuploidy) both *in vitro* and *in vivo*	No valid evidence for gene mutation in any test; no evidence for chromosome aberrations in humans and equivocal findings elsewhere
Structure–activity relationships	Positive for structural alerts associated with genetic activity	No structural alerts for glyphosate or AMPA suggesting genotoxicity
DNA binding	Agent or breakdown product are typically electrophilic and exhibit direct DNA binding	No unequivocal evidence for electrophilic properties or direct DNA binding by glyphosate or AMPA
Consistency	Test results are highly reproducible both *in vitro* and *in vivo*	Conflicting and/or non-reproducible responses in the same test or test category both *in vitro* and *in vivo*
Response kinetics	Responses are dose dependent over a wide range of exposure levels	Many positive responses do not show significant dose-related increases
Susceptibility to confounding factors (e.g. cytotoxicity)	Responses are typically found at nontoxic exposure levels	Positive responses typically associated with evidence of overt toxicity

AMPA: aminomethylphosphonic acid; GBF: glyphosate-based formulation.

categories (mutation, chromosomal effects) associated with carcinogen prediction. As additional support for the Panel's WoE conclusion, Table 10 provides a comparison between a set of characteristics associated with confirmed genotoxic carcinogens (Bolt et al. 2004; Petkov et al. 2015) and the genotoxic activity profiles for glyphosate, AMPA, and GBFs. There is virtually no concordance between the two sets of characteristics.

Beyond the standard genetic toxicity assays, IARC concluded for humans exposed to GBFs that there was positive evidence of DNA breakage as determined using the comet assay (Paz-y-Miño et al. 2007), negative induction of chromosomal aberrations (Paz-y-Miño et al. 2011), and positive induction of MN (Bolognesi et al. 2009). These papers were critically reviewed by the Expert Panel and were found to be deficient as evidence for GBF genetic effects for many reasons (e.g. identification of cells scored for comets, inconsistent observations, uncertainties with respect to "negative controls," lack of statistical significance, and lack of effect relative to self-reported exposure). In addition to questions about the significance of the comet endpoint there is also a lack of scientific consensus regarding the relevance of MN found in exposed humans (Speit 2013; Kirsch-Volders et al. 2014). Importantly, for the Bolognesi study, increases in MN were not significantly correlated with self-reported GBF spray exposure and were not consistent with application rates. The Expert Panel concluded that there was little or no reliable evidence produced in these studies that would support a conclusion that GBFs, at levels experienced across a broad range of end-user exposures, poses any human genotoxic hazard/risk.

With respect to oxidative stress and genotoxic potential of glyphosate and its formulations, it is noted that many more oxidative stress studies are available for GBFs than for glyphosate or AMPA. A higher proportion of the GBF studies show evidence of oxidative stress. This might be consistent with induction of oxidative stress by GBF components such as surfactants. IARC's statement that there is strong evidence supporting oxidative stress from AMPA seems to result from glyphosate and particularly GBF results rather than AMPA results. In fact, oxidative stress studies of AMPA are very limited. The paucity of cited data does not seem to justify a conclusion of strong evidence for oxidative stress induction by AMPA.

One mechanism connecting oxidative stress to induction of carcinogenicity is oxidative damage to DNA and the generation of mutagenic lesions. Most of the endpoints used in oxidative stress studies cited by IARC are indirect response endpoints and the number of studies examining direct oxidative DNA damage are very few and presented mixed results. Further, research on oxidative stress-induced genotoxicity suggests that it is often a secondary response to toxicity and characterized by a threshold (Pratt & Barron 2003). Comparison of GBF oxidative stress study results with predicted human exposure levels of less than 0.064 mg/kg bw/day, suggests that it is improbable that GBFs would induce levels of oxidative stress likely to exceed endogenous detoxication capacities.

The most appropriate conclusion supported by the oxidative stress data is, based on a WoE approach, that there is no strong evidence that glyphosate, GBFs, or AMPA produce oxidative damage to DNA that would lead to induction of endpoints predictive of a genotoxic hazard or act as a mechanism for the induction of cancer in experimental animals or humans.

A thorough WoE review of genotoxicity data does not indicate that glyphosate, GBFs, or AMPA possess the properties of genotoxic hazards or genotoxic mechanisms of carcinogenesis.

Discussion and conclusions

Four Expert Panels conducted detailed reviews of glyphosate exposure, animal carcinogenicity, genotoxicity, and epidemiologic studies. With respect to exposure, even when using a number of worst-case assumptions, systemic doses of glyphosate in human applicators, bystanders, and the general public are very small. Exposures of the general public are three or more orders of magnitude less than the US EPA's RfD (1.75 mg/kg/day) as well the ADIs established by JMPR (1 mg/kg/day) and EFSA (0.5 mg/kg/day). The RfD is the allowable limit of daily exposure derived from toxicity studies, and even in the most exposed applicators (90th centile) the systemic dose was estimated at 20-fold less that the RfD. Exposures to the public are in the range of 0.00001–0.001 mg/kg bw/day while occupational exposures can range up to 0.01 mg/kg

bw/day. Systemic exposures are even lower than the reported ranges since oral and dermal absorption of glyphosate is low.

With respect to the animal cancer bioassay data, the Expert Panel conducted a thorough overall WoE evaluation that considered a much wider range of studies than IARC, all of which met Good Laboratory Practice (GLP) guidelines and were submitted to support glyphosate Annex I renewal in the European Union. These studies provided evidence that neoplasms naturally occurring in rodents are widely represented in non-exposed animals, as well as those exposed to doses well below those that might be expected in regulatory studies. The pattern of occurrence of these tumors was found to be inconsistent across and within species and no "novel" neoplasms appeared; progression of non-neoplastic to neoplastic lesions also was not seen. Further, the comparatively large number of studies performed would be expected to generate several numerical imbalances by chance. In fact, Haseman (1983) has estimated that the overall false positive rate for animal bioassays that tested both sexes in two species, because of multiple comparisons, corresponds to 7–8% significance level for the study as a whole; the US Food and Drug Administration has estimated that the overall rate can approach 10%.

After review of all available glyphosate rodent carcinogenicity data, the Panel concludes:

- The mouse renal neoplastic effects are not associated with glyphosate exposure, because they lack statistical significance, consistency, specificity, a dose-response pattern, plausibility, and coherence;
- The association of hemangiosarcomas in the livers of mice is weak, lacks consistency, and there was no dose-response effect;
- The association of pancreatic islet-cell adenomas in male SD rats is weak, not seen in the majority of rat studies, lacks a dose-response pattern (the highest incidence is in the low dose followed by the high dose), plausibility and pre-neoplastic/malignant effects;
- In one study, the significant positive trend in the incidence of hepatocellular adenomas in male rats did not materialize, no progression to malignancy was evident and no glyphosate-associated pre-neoplastic lesions were present;
- In one study, the significant positive trend in the incidence of thyroid C-cell adenomas in female rats did not materialize, the adenomas were only slightly increased in mid- and high doses, and there was no progression to malignancy.

Overall, extensive reviews of the genotoxicity of glyphosate, AMPA, and GBFs that were available prior to the development of the IARC Glyphosate Monograph all support a conclusion that glyphosate (and related materials) is inherently not genotoxic. Further, evidence indicative of an oxidative stress mechanism of carcinogenicity is largely unconvincing. The Expert Panel concluded that there is no new, valid evidence presented in the IARC Monograph that would provide a basis for altering these conclusions.

Lastly, the Expert Panel's review of the glyphosate epidemiologic literature and the application of commonly applied causal criteria did not indicate a relationship with glyphosate exposure and NHL. In addition, the Panel considered the evidence for MM to be inadequate to judge a relationship with glyphosate. The extremely large margin of safety found in exposure monitoring studies is considered to be supportive of these conclusions.

In summary, the totality of the evidence, especially in light of the extensive testing that glyphosate has received, as judged by the Expert Panels, does not support the conclusion that glyphosate is a "probable human carcinogen" and, consistent with previous regulatory assessments, the Expert Panels conclude that glyphosate is unlikely to pose a carcinogenic risk to humans.

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Declaration of interest

The employment affiliation of the authors is as shown on the cover page. However, it should be recognized that each individual participated in the review process and preparation of this paper as an independent professional and not as a representative of their employer.

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Intertek (previously Cantox) is a consultancy firm that provides scientific and regulatory advice, as well as safety and efficacy evaluations for the chemical, food, and pharmaceutical industries. While Intertek has not previously worked on glyphosate-related matters for the Monsanto Company, previous employees (Ian Munro, Barry Lynch) of Cantox, have worked in this capacity. These employees of Cantox, and Gary M. Williams, prepared a safety and risk assessment, including the carcinogenicity, of Roundup herbicide (glyphosate), which was published in 2000 (Williams et al. 2000).

Gary M. Williams, Sir Colin Berry, David Brusick, João Lauro Viana de Camargo, Helmut A. Greim, David J. Kirkland, Keith R. Solomon, and Tom Sorahan have previously served as independent consultants for the Monsanto Company on the European Glyphosate Task Force. John Acquavella and Larry D. Kier have also served as independent consultants and were previously employees of the Monsanto Company. John Acquavella was employed by Monsanto between the years 1989 and 2004 while Larry D. Kier was employed between 1979 and 2000. David Garabrant serves on a scientific advisory board to Dow Agro Sciences, which markets pesticides including glyphosate, and has consulted on behalf of Bayer Corp. on litigation matters concerning glyphosate and leukemia. Gary Williams and Tom Sorahan have consulted for Monsanto on litigation matters involving glyphosate. Tom Sorahan has received consultancy fees and travel grants from Monsanto Europe SA/NV as a member of the European Glyphosate Toxicology Advisory Panel and participated in the IARC Monograph Meeting for volume 112, as an Observer for the Monsanto Company. Douglas L. Weed has consulted on litigation matters concerning Monsanto that did not involve glyphosate. Marilyn Aardema, Michele M. Burns, Gary Marsh, and Ashley Roberts have not previously been employed by the

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REVIEW ARTICLE

OPEN ACCESS

Glyphosate in the general population and in applicators: a critical review of studies on exposures

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ABSTRACT

The recent classification of glyphosate as a probable human carcinogen by the International Agency for Research on Cancer (IARC) was arrived at without a detailed assessment of exposure. Glyphosate is widely used as an herbicide, which might result in exposures of the general public and applicators. Exposures were estimated from information in the open literature and unpublished reports provided by Monsanto Company. Based on the maximum measured concentration in air, an exposure dose of 1.04×10^{-6} mg/kg body mass (b.m.)/d was estimated. Assuming consumption of surface water without treatment, the 90th centile measured concentration would result in a consumed dose of 2.25×10^{-5} mg/kg b.m./d. Estimates by the Food and Agriculture Organization of the United Nations (FAO) of consumed doses in food provided a median exposure of 0.005 mg/kg b.m./d (range 0.002–0.013). Based on tolerance levels, the conservative estimate by the US Environmental Protection Agency (US EPA) for exposure of the general population *via* food and water was 0.088 mg/kg b.m./d (range 0.058–0.23). For applicators, 90th centiles for systemic exposures based on biomonitoring and dosimetry (normalized for penetration through the skin) were 0.0014 and 0.021 mg/kg b.m./d, respectively. All of these exposures are less than the reference dose and the acceptable daily intakes proposed by several regulatory agencies, thus supporting a conclusion that even for these highly exposed populations the exposures were within regulatory limits.

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Introduction

The recent classification of glyphosate as a probable human carcinogen by the International Agency for Research on Cancer (IARC 2015) has generated considerable interest, particularly as the IARC classification was arrived at without a detailed assessment of risk to applicators and the general public. Glyphosate is widely used for control of weeds in agriculture, forestry, and in the management of public and private landscapes. These uses might result in exposures of the general public as well as applicators. Unfortunately, the IARC monograph merely focused on the potential hazards of glyphosate and not on the risks. Exposure is a critical component of risk assessment and, without measured values; it is difficult to provide guidance on the appropriate uses of glyphosate or, for that matter, any pesticide. It is also not possible to properly assess toxicity and hazard data for relevance to humans and the environment. As per their mandate, none of the IARC evaluations characterize exposures analytically or in the context of risk; the monograph on glyphosate (IARC 2015) summarizes several exposure studies from the open literature, but does not use these values to estimate risks. This is different from the approach used by most regulatory agencies such as the US EPA, the Food and Agricultural Agency (FAO) of the United Nations, and the European Food Safety Agency (EFSA) where exposures are compared to Reference Doses (RfDs) or Acceptable Daily Intake (ADIs).

There are several sources of exposure of humans to glyphosate in the environment. These are: air, water, application

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to crops and target weeds, and food. The following sections are an analysis of exposures of humans to glyphosate from these sources. Data for these exposures were obtained from papers published in the open literature and from unpublished reports provided by the Monsanto Company. These sources of information are listed in the references and summary data are provided in the Supplemental information (SI).

Methods

Unpublished reports of studies on exposure to glyphosate in applicators were provided by the Monsanto Company and covered uses in agriculture and forestry. Other data on exposures were obtained from the open literature as a result of searches in PubMed®, references in reviews, and Google Scholar®. These papers and reports were grouped into sources of exposures and the data analyzed as described below.

Air

Only one paper reported concentrations of glyphosate in air. In a study conducted in Iowa, Mississippi, and Indiana in 2007 and 2008, concentrations of glyphosate and its major environmental degradation, aminomethylphosphonic acid (AMPA) were measured in air and precipitation (Chang et al. 2011). Detections of AMPA were infrequent and the concentrations were small. These are not discussed further. The frequency of detection of glyphosate ranged from 60 to 100% in air and rainwater. Concentrations in air ranged from <0.01 to 9.1 ng/m^3, while those in rain were from <0.1 to 2.5 µg/L. Unless rainwater was collected as drinking water, this would be an incomplete pathway for exposure of humans. Once in contact with soil, exposures would be via surface waters (see below). Concentrations in air were seasonal and the sources were likely associated with application to crops in the growing season. For estimation of human exposure, it was assumed that there was total absorption of glyphosate from the air into the body of a 70 kg human breathing 8 m^3 air (half a day for an adult, US EPA 2009). These values were then used to calculate the systemic dose, based on a worst-case assumption of 100% uptake *via* the respiratory tract.

Water

Glyphosate can enter surface waters through use on aquatic weeds, runoff from sprayed soils, and from drift of spray. Glyphosate is very soluble in water and, although it binds strongly to soils and sediments, small concentrations have been measured on surface waters in the United States. These measurements are part of the US Geological Survey (USGS) National Water-Quality Assessment (NAWQA) program (USGS 2015), which has been in place since the 1980s. Glyphosate was added to the large range of analytes measured in surface water in 2002. These data were downloaded from the NAWQA data warehouse (USGS 2015) and then sorted by concentration. All values measured across the US between 2002 and 2014 were pooled for the analysis. Where concentrations were less than the level of detection (0.02 µg glyphosate acid equivalents (a.e.)/L), these values were substituted with a dummy value of "zero". The values were ranked from the smallest to the largest and a cumulative frequency distribution was derived. These values were processed using the Weibull formula to estimate ranks and plotted on a log-probability scale (Solomon and Takacs 2002). The 90th centile values were calculated from the raw data using the Excel function <=percentile>. Systemic dose was estimated from the assumption of consumption of 2 L of water per day by a 70 kg human with 20% absorption from the gastrointestinal (GI) tract (EFSA 2015). Although chlorine and ozone are highly effective for removing glyphosate and AMPA during purification of drinking water (Jönsson et al. 2013), it was assumed that treatment did not remove any glyphosate. The estimated concentrations are thus a worst-case.

Food and bystanders

Several studies have measured concentration of glyphosate in "bystanders" and people not involved in application of glyphosate. Bystanders are presumable exposed *via* food, water, and air (see above). It is also assumed that bystanders are exposed on a daily basis through the environment and/or food and drinking water, and that these exposures are constant and not episodic as in an applicator. Here, a single daily sample of urine is a reasonable surrogate for daily exposures, although uncertainty would be reduced with more frequent samples and analysis of total daily urinary output. Several of these studies were critically reviewed in 2015 (Niemann et al. 2015). This review was thorough, but the strengths of the methods of the original studies were variable. In addition, the authors did not correct for incomplete excretion of glyphosate (95%) as has been done for the applicator studies. In a study of farm and non-farm households in Iowa (Curwin et al. 2007), urine samples were analyzed from 95 adults and 117 children. A study in Europe (Mesnage et al. 2012) measured exposures in a farm family (two adults and three children). A report on the analysis of urine of 182 people from 18 countries (Hoppe 2013) provided data on concentrations in urine. In another study, urine concentrations of 40 male and female German students were measured (Markard 2014). The original study was in German and the value used here for the systemic dose is from the review of Niemann et al. (2015). A study using enzyme linked immunosorbent assay (ELISA) analysis with an unstated level of quantitation (LOQ) was used to measure the concentrations of glyphosate in samples of urine from more than 300 individuals in the EU (most from Germany) (Krüger et al. 2014). A report of a study in the US on 35 individuals using an ELISA analysis (Honeycutt and Rowlands 2014) provided data from which a systemic dose of glyphosate was estimated.

Where the systemic dose was calculated, it was used. Where dietary exposures were provided, the urinary concentration was used to calculate the systemic dose on the assumption of 2 L of urine per day and a 60 kg person (Niemann et al. 2015).

Under the auspices of the Food and Agricultural Organization of the United Nations, the Joint Meeting on Pesticide Residues (JMPR) conducts routine assessments of residues of pesticides in food (JMPR 2014). These are

evaluated in relation to diets in various regions of the world and exposure *via* food compared to an ADI. In 2013, the JMPR reviewed dietary exposures to glyphosate, its major metabolites, and breakdown products (N-acetyl glyphosate, AMPA, and N-acetyl AMPA) and calculated the international estimated daily intakes (IEDI) of glyphosate for 13 regional food diets (JMPR 2014). These IEDIs were based on estimated mean residues from supervised trails under normal or good agricultural practice. These values were for a 60 kg person but were used without modification.

The US Environmental Protection Agency (US EPA) has calculated exposures to glyphosate using the Dietary Exposure Evaluation Model (DEEM, ver 7.81), which is based on tolerance levels for all commodities and modeled estimates of exposures from food and drinking water for the overall US population (US EPA 2012).

There is some uncertainty in all of these studies and approaches. All of the monitoring studies used relatively few participants (<300), which increases uncertainty and lack of raw data in most studies does not allow variance to be fully characterized. Modeling approaches (US EPA and JMPR) based on maximum residue limits and assumptions of good agricultural practices are also subject to uncertainty; however, the assumptions used are more likely to result in overestimation. However, proportion of foods consumed is based on the statistical analyses of diets and this does incorporate, but not quantify, uncertainty.

Applicators

A relatively large number of studies on exposures of applicators to glyphosate have been conducted (see SI for a full list). Older studies tended to use passive dosimetry, either as whole-body dosimeters or patches. Some of the studies with dosimeters used tracers (dyes or other surrogates) and others analyzed dosimeters for glyphosate itself. Some more recent studies used biological monitoring and some a mixture of biological monitoring and dosimeter-patches. For compounds, such as glyphosate, where the excretion kinetics is well understood, biological monitoring provides a measure of the actual amount of the chemical in the body. For this reason, data from these studies are most appropriate for risk assessment. However, data from dosimetry studies can be used to estimate systemic dose. This allows comparison of exposures from different studies to a benchmark for exposure i.e. the reference dose (RfD) or ADI.

For studies using dosimetry, the normalization to systemic dose was conducted using the procedure outlined in Table 1. This was done for the dosimetry studies listed in SI Table 1. The estimated systemic doses were ranked from smallest to largest and a cumulative frequency distribution was derived. These values were plotted on a log-probability scale as above. The 90th centile values were calculated from the raw data using the Excel® function <=percentile>.

Where an applicator makes a single application, the systemic dose of glyphosate can be estimated from the total amount of glyphosate excreted in the urine over the four or five days following and including the day of application (Acquavella et al. 2004). Glyphosate is rapidly excreted and does not bioaccumulate. If applications are conducted every day, the amount excreted each day provides a time-weighted average for daily exposures. Because glyphosate is applied infrequently in normal agricultural practice, the assumption of a single initial exposure is appropriate for risk assessment.

The procedure of normalization for biomonitoring studies is complicated by the fact that many studies reported concentrations of glyphosate that are less than the LOQ, even on the day of application (d-0), when exposures would be expected to be greatest. Similarly, even if residues were detected on d-0, those on subsequent days might have values less than the LOQ. The common practice of using half the level of detection as a default value might be acceptable for the first observation day, but this fails to account for excretion that would reduce the amount in the body on each successive day. Use of half the LOQ on each day would grossly overestimate the systemic dose. Because of this, normalization of systemic doses was modeled using excretion kinetics and followed the steps outlined in Table 2.

If concentrations in urine are > LOQ for one or more days, the actual elimination rate for the individual can be used to correct for days where concentration is < LOQ. Unless already carried out in the study itself, these corrections were applied to the data in SI Table 2.

Because raw data were available for the studies on applicators, uncertainty could be considered. Total number of participants was large (249, See SI Table 2) and range of the values provided the upper and lower bounds of uncertainty. To be conservative, the 90th centiles of the data were used to characterize reasonable worst-case exposures.

Normalization of the RfD and ADI for systemic dose

Regulatory agencies set allowable limits for consumption of residues of glyphosate exposure based on toxicity studies. The US EPA RfD is 1.75 mg/kg body mass (b.m.)/day (US EPA 2012). The ADI for JMPR/WHO is 1 mg/kg b.m./d (JMPR 2014), while the ADI used by EFSA is 0.5 mg/kg b.m./d (EFSA 2015). In a recent review (summary published on 16 May 2016),

Table 1. Procedure for normalization of dosimetry data to estimate systemic dose.

Step	From		To	Explanation
1	Total residue on patches μg/cm^2)	to	Potential body exposure (μg)	2.1 m^2 surface area for a 70 kg male (US EPA 2009)
2	Potential body exposure (μg)	to	Actual body exposure (μg)	Measured penetration through clothing or default of 10%
3	Actual body exposure (μg)	to	Systemic body exposure (μg)	1% dermal penetration (from the value used by EFSA 2015)
4	Systemic body exposure (μg)	to	Systemic dose (mg/kg body weight/day)	70 kg adult

Table 2. Procedure for normalization of biomonitoring data to estimate systemic dose of glyphosate.

Step	Data	Action
1	LOD = 10 µg/kg urine	Assume half the LOD = 5 µg/kg
2	Adjust estimated dose to amount of urine	Multiple kg urine produced on day by 1/2 LOD
3	D-0 value amount estimated	C_0 amount
4	D-1 value estimated from remainder of d-0 concentration after excretion	Elimination rate constant (k) of 0.86 d^{-1} from (Acquavella et al. 2004) use $C_t. = C_0 \times e^{-kt}$
5	D-2 value estimated from remainder of d-1 concentration after excretion	
6	D-3 value estimated from remainder of d-2 concentration after excretion	
7	D-4 value estimated from remainder of d-3 concentration after excretion	
8	D-5 value estimated from remainder of d-4 concentration after excretion	
9	Sum of amounts for each day of urine collected	
10	Correction for monitoring period from elimination rate constant and number of days	For example, 99% for 5 d, divide by 0.99
11	Correction for incomplete excretion (95%)	Based on observations in TK studies in monkeys, which showed that 95% of total systemic dose was excreted *via* urine (Wester et al. 1991), divided by 0.95
12	Correction for dosimeters, if used	Increase dose by percentage of body area represented by the dosimeters
13	Correction for hand wash or gloves, if used	Increase dose by percentage of body area represented by hands
14	Calculate systemic dose	Divide total systemic dose by body mass

C_0: initial concentration; C_t: concentration at time t; LOD: level of detection; TK: toxicokinetic.

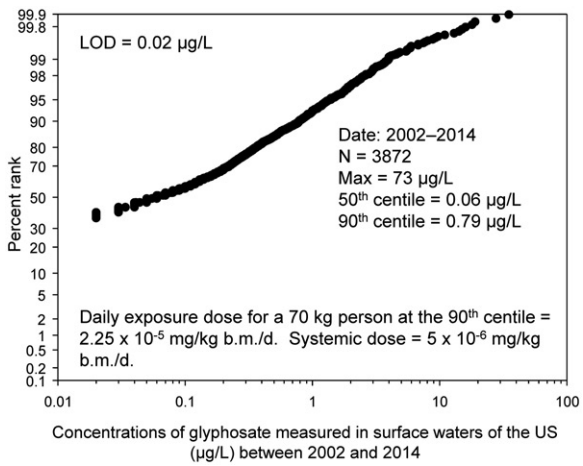

Figure 1. Distribution of concentrations of glyphosate measured in surface waters across the US.

JMPR (2016) has reaffirmed their ADI of 1 mg/kg b.m./d. These values are suitable for comparison to the dietary intake, but for comparison to systemic doses as estimated from biological monitoring (urinary excretion), the ADIs and RfD were divided by five to account for only 20% absorption from the GI tract (EFSA 2015). These normalized values are 0.35, 0.2, and 0.1 mg/kg b.m./d, for US EPA, JMPR, and EFSA, respectively.

Results

Air

Based on the above assumptions of respiratory volume and total absorption, inhaling glyphosate in air at the maximum measured concentration would result in an exposure dose of 1.04×10^{-6} mg/kg b.m./d. This is about five orders of magnitude less than the systemic ADI proposed by EFSA (2015).

Water

The cumulative frequency distribution of concentrations of glyphosate measured in surface waters of the US are shown in Figure 1. The 90th centile was 0.79 µg/L. The maximum concentration measured was 73 µg/L. Consumption of 2 L of drinking water by a 70 kg person at the 90th centile concentration is estimated to result in a consumed dose of 2.25×10^{-5} mg/kg b.m./d, more than four orders of magnitude less than the EFSA ADI.

Food and bystanders

Estimates of the systemic dose resulting from exposures of bystanders and the general public to glyphosate are shown in Table 3. All of these systemic doses are more than 150-times less than the EFSA ADI, normalized for reduced uptake from the gut.

Based on the estimates of daily intake from the FAO/JMPR, the minimum IEDI was 124 µg/person/d, the median was 301, and maximum was 762 (JMPR 2014). These values were normalized to a 60 kg person (0.002, 0.005, and 0.013 mg/kg b.m./d, respectively) for comparison to the ADI. Median exposures are 100-times less than the ADI suggested by EFSA.

The dietary exposure of the general population in the US was estimated by US EPA to be 0.088 mg/kg b.m./d and the range of values was from 0.058 to 0.23 mg/kg b.m./d across a range of age-groups from adults to toddlers. These values are all less than the ADI suggested by EFSA.

Applicators

For the applicator studies, the corrections were applied as in Table 1 or Table 2 and the results are presented graphically in Figure 2. Raw data are provided in SI Tables 1 and 2.

Table 3. Summary of exposures to glyphosate in bystanders and the general public.

Study	Source of exposure	Urinary concentration (μg/L)		Systemic dose (mg/kg b.m./d)		Comment
		Greatest mean	Maximum	Greatest mean	Maximum	
Table 2 from Curwin et al. 2007	Presumably food and water from non-farm households in Iowa	2.7	9.4	0.00009	0.00031	Highest mean and max was in non-farm children
Table 3 from Curwin et al. 2007	Bystanders from farm households in Iowa	2.1	–	0.00007	–	Highest median was in farm children. Max not reported.
Mesnage et al. 2012	Bystander, farm family of five	–	2	–	0.00007	Maximum concentration in child
Hoppe 2013	Presumably food and water	0.82	1.82	0.000027	0.000061	Highest mean was in samples from Malta
Markard 2014	Presumably food and water	–	0.65	–	0.000022	Maximum concentration
Krüger et al. 2014	Presumably food and water	–	5	–	0.00017	Maximum concentration
Honeycutt and Rowlands 2014	Presumably food and water	–	18.8	–	0.00063	Maximum concentration

Systemic dose (mg/kg b.m./d): Urinary concentration (μg/L) × 2 L urine/day ÷60 kg body mass ×1000. b.m.,.

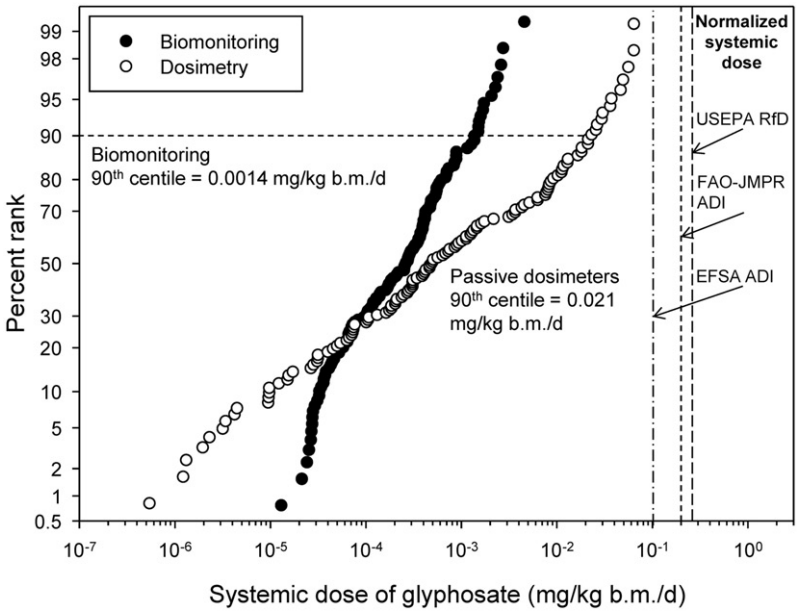

Figure 2. Systemic doses of glyphosate measured in exposure studies conducted in applicators.

The range of values for systemic doses measured in the dosimeter studies (90th centile =0.021 mg/kg b.m./d) was greater than in the biomonitoring studies (90th centile =0.0014 mg/kg b.m./d). Given the corrections applied to the data, this is surprising; however, there are a number of assumptions used in the normalization of the systemic doses that might result in overestimation of exposure. These are likely in the amount of absorption though skin and the penetration of clothing. The assumption of 1% penetration through the skin is greater than the value of 0.7% suggested from observations in an *in vitro* model with human skin (Bo Nielsen et al. 2009). The 90th centile in the dosimetry studies was 0.021 mg/kg b.m./d; about five-times less than the systemic EFSA ADI.

The range of values for the systemic doses determined by biomonitoring was smaller than for the passive dosimeters and more accurately reflects the true exposures. The 90th centile was 0.0014 mg/kg b.m./d; about 70-times less than the systemic EFSA ADI.

Conclusions

Even when using a number of reasonable worst-case assumptions, systemic doses of glyphosate in human applicators, bystanders, and the general public are small. Exposures to glyphosate in the general public are less than EFSA's ADI. The same conclusion applies to applicators. As an overall summary, exposures and ADIs are compared graphically in Figure 3. It should be noted that the ADIs and RFDs used in this assessment are derived from the most sensitive response in long-term feeding studies in the most sensitive laboratory test species and that an uncertainty factor is applied to these values. Furthermore, the biomonitoring exposures measured in applicators aggregate all sources of exposures (air, food, water, and dermal contact) and are still less than the most conservative ADI. Based on the current RfDs and ADIs, there is no hazard and no intolerable risk from exposure to glyphosate *via* its normal use in agriculture and management of weeds in landscapes.

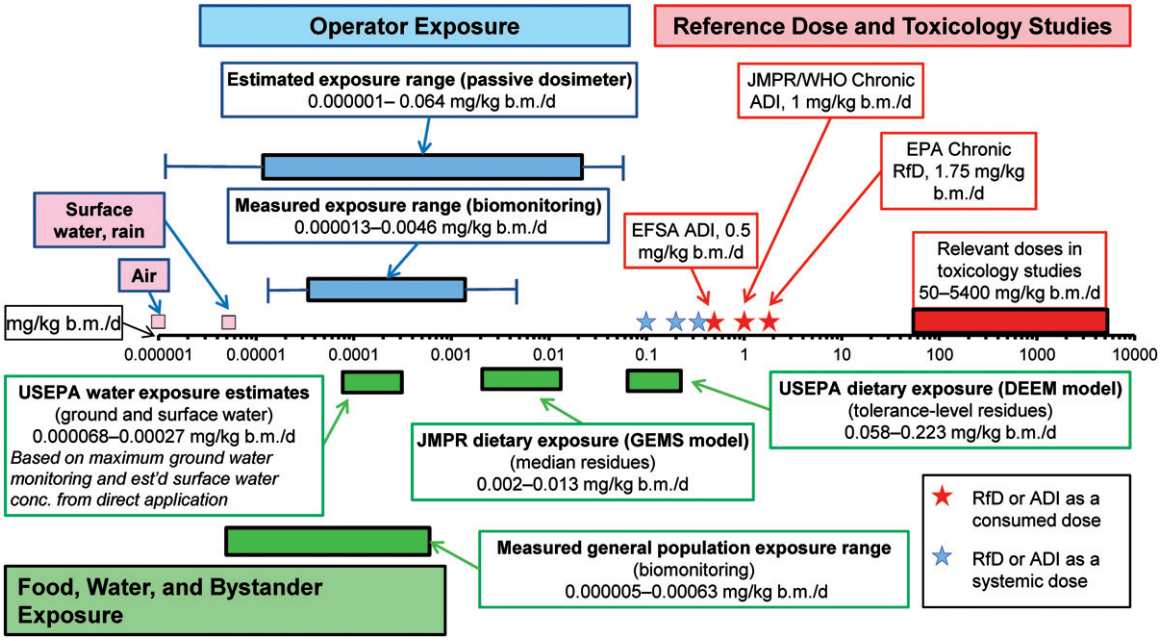

Figure 3. Illustration of measured and estimated exposures to glyphosate in applicators and the general public from various sources. Solid horizontal bars show 10–90th centiles, whiskers show minimum and maximum.

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Declaration of interest

The employment affiliation of the author is shown on the cover page. However, it should be recognized that the author participated in the review process and preparation of this paper as an independent professional and not as a representative of his employer. Keith R. Solomon previously served as an independent consultant for the Monsanto Company on the European Glyphosate Task Force. KS has not been involved in any litigation procedures involving Monsanto Company and glyphosate. KS's recruitment and evaluation of the data was organized and conducted by Intertek Scientific & Regulatory Consultancy (Intertek). KS acted as a consultant for Intertek. Intertek (previously Cantox) is a consultancy firm that provides scientific and regulatory advice, as well as safety and efficacy evaluations for the chemical, food and pharmaceutical industries.

While Intertek Scientific & Regulatory Consultancy has not previously worked on glyphosate related matters for the Monsanto Company, previous employees of Cantox had worked in this capacity. Funding for this evaluation was provided by the Monsanto Company which is a primary producer of glyphosate and products containing this active ingredient. Neither any Monsanto company employees nor any attorney reviewed any of the Expert Panel's manuscripts prior to submission to the journal.

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Supplemental material

Supplemental material for this article is available online here.

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Glyphosate epidemiology expert panel review: a weight of evidence systematic review of the relationship between glyphosate exposure and non-Hodgkin's lymphoma or multiple myeloma

John Acquavella, David Garabrant, Gary Marsh, Tom Sorahan & Douglas L. Weed

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REVIEW ARTICLE

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Glyphosate epidemiology expert panel review: a weight of evidence systematic review of the relationship between glyphosate exposure and non-Hodgkin's lymphoma or multiple myeloma

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ABSTRACT

We conducted a systematic review of the epidemiologic literature for glyphosate focusing on non-Hodgkin's lymphoma (NHL) and multiple myeloma (MM) – two cancers that were the focus of a recent review by an International Agency for Research on Cancer Working Group. Our approach was consistent with Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines for systematic reviews. We evaluated each relevant study according to *a priori* criteria for study quality: adequacy of study size, likelihood of confounding, potential for other biases and adequacy of the statistical analyses. Our evaluation included seven unique studies for NHL and four for MM, all but one of which were case control studies for each cancer. For NHL, the case-control studies were all limited by the potential for recall bias and the lack of adequate multivariate adjustment for multiple pesticide and other farming exposures. Only the Agricultural Health (cohort) Study met our *a priori* quality standards and this study found no evidence of an association between glyphosate and NHL. For MM, the case control studies shared the same limitations as noted for the NHL case-control studies and, in aggregate, the data were too sparse to enable an informed causal judgment. Overall, our review did not find support in the epidemiologic literature for a causal association between glyphosate and NHL or MM.

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Introduction

The epidemiologic literature for glyphosate was reviewed recently as part of a multi-disciplinary scientific review by the International Agency for Research on Cancer (IARC 2015). In the aftermath of the IARC review and the designation of glyphosate as probably carcinogenic to humans, the Monsanto Company requested expert reviews of the glyphosate literature in several technical areas, including epidemiology. IARC's working group concluded that there was limited epidemiologic evidence[1] in human studies for the carcinogenicity of glyphosate, based on a positive association observed for non-Hodgkin's lymphoma (NHL). The panel also noted that excesses had been observed for multiple myeloma (MM) in three studies, but felt these results were less reliable because of small numbers of cases in the available studies and the

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Table 1. Relevant studies for glyphosate review: non-Hodgkin's lymphoma (NHL) and multiple myeloma (MM).

Author, year	Study location(s)	Study design	More recent analysis	Outcome
Cantor et al. 1992	Iowa + Minnesota	Case-control	De Roos et al. 2003	NHL
Nordstrom et al. 1998	Sweden	Case-control	Hardell et al. 2002	HCL
Hardell & Eriksson 1999	Sweden	Case-Control	Hardell et al. 2002	NHL excluding HCL
McDuffie et al. 2001	Canada	Case-control	n/a	NHL
Hardell et al. 2002	Sweden	Case-control (pooled)	n/a	NHL + HCL
De Roos et al. 2003	Nebraska Iowa/Minnesota Kansas	Case-control (pooled)	n/a	NHL
De Roos et al. 2005	Iowa, North Carolina	Cohort	n/a	NHL, MM
Eriksson et al. 2008	Sweden	Case-control	n/a	NHL
Orsi et al. 2009	France	Case-control	n/a	NHL, MM
Hohenadel et al. 2011	Canada	Case-control	Extension of McDuffie et al. 2001	NHL
Cocco et al. 2013	Czech Republic, France, Germany, Ireland, Italy, Spain	Case-control	n/a	B-cell lymphoma
Brown et al. 1993	Iowa	Case-control	n/a	MM
Landgren et al. 2009	Iowa North Carolina Minnesota	Prevalence Case-control	n/a	MGUS
Pahwa et al. 2012	Canada	Case-control	Kachuri et al. 2013	MM
Kachuri et al. 2013	Canada	Case-control	n/a	MM
Sorahan 2015	Iowa, North Carolina	Cohort	Reanalysis of De Roos et al. 2005	MM

HCL: hairy cell leukemia; MGUS: monoclonal gammopathy of undetermined significance.

related inability to adjust findings for other pesticide and farming exposures. Lastly, the panel concluded that there was no epidemiologic evidence of a relationship for other cancer sites with respect to glyphosate exposure.

In this epidemiology expert panel review, we focused on the possible relationship between glyphosate exposure and two cancers that were the focus of the IARC epidemiology review: NHL and MM. The focus of our review was qualitative. That is, we evaluated the published evidence according to widely accepted validity considerations and criteria for causality. When there were two or more publications with overlapping populations, we concentrated on the most recent publication noting the relationship to a previous publication(s) (see Table 1). Herein, in succeeding sections, we have presented our evaluation approach, reviewed the key validity issues for epidemiologic studies of pesticides, detailed some statistical considerations pertinent to the glyphosate literature, critically evaluated published studies, and, lastly, provided an overall weight of evidence assessment of the epidemiologic evidence for causality between glyphosate and NHL or MM.

Methods

The approach we took was informed by and consistent with the PRISMA guidelines for systematic reviews (Moher et al. 2009), standard approaches to critically evaluating epidemiologic studies (Aschengrau & Seage 2003a,b; Sanderson et al. 2007) and well-recognized interpretative methods – e.g. the criteria-based methods of causal inference (Hill 1965, 1971) – sometimes referred to as "weight of evidence" methods (Weed 2005). With this approach in mind, we address the following questions:

1. Does the current published epidemiologic evidence establish a causal relationship between glyphosate exposure and NHL?
2. Does the current published epidemiologic evidence establish a causal relationship between glyphosate exposure and MM?

Other types of scientific evidence are often evaluated when making causal determinations, including data on human exposure as well as animal studies and studies on mechanism. Since exposure assessment is critical for the validity of occupational epidemiologic studies and biologic plausibility is informed by presumed dose, the former were considered in our overall assessments.

Literature search and included/excluded published papers

A systematic search of the medical literature was performed to identify all analytic epidemiological studies that have examined the possible relationships between exposure to glyphosate and NHL and MM. The aim was to include all such publications – case control studies, cohort studies and pooled analyses – published to the present. In this process, other publications are typically identified, such as reviews, commentaries, methodological investigations, letters to the editor and case reports (or case series). Our primary concern here, however, was the evaluation of the published analytical epidemiological studies of glyphosate and either NHL or MM. To the extent that other types of publications inform our assessment, those papers will be cited in this report. The so-called "gray literature[2]" was not reviewed.

Medline (PubMed) and TOXLINE were searched for English-language publications (with no time constraints) as follows:

a. PubMed: (2 August 2015): search terms: "glyphosate" and "cancer" ($n = 31$);
b. TOXLINE: (2 August 2015): search terms: "glyphosate" and "cancer" ($n = 48$);
c. PubMed: (13 August 2015): search terms: "herbicide" and "cancer" and "lymphoma" and "epidemiology" ($n = 153$);
d. PubMed: (24 August 2015): search: "herbicide" and "cancer" and "multiple myeloma" and "epidemiology" ($n = 38$);

Table 2. Results for glyphosate: Non-Hodgkin's lymphoma (NHL).

Author, year (study design)	# cases, controls total or exposed	OR/RR (95% CI)	Multivariate adjustments	Outcome
McDuffie et al. 2001	517, 1506 [total]			NHL
(case-control)	51, 133	Any use OR = 1.2 (95% CI 0.8, 1.7)	Age, province, medical conditions	
	28, 97	≤2 days/year OR = 1.0 (95% CI 0.6, 1.6)	Age, province	
	23, 36	>2 days/year OR = 2.1 (95% CI 1.3, 2.7)		
Hardell et al. 2002	515, 1141 [total]			NHL + HCL
(case-control)	8, 8	Any use OR = 3.0 (95% CI 1.1, 8.5)	None	
	8, 8	Any use OR = 1.9 (95% CI 0.6, 6.2)	Multivariate (unspecified)	
De Roos et al. 2003	650, 1933 [total]			NHL
(case-control)	36, 61	Any use OR = 2.1 (95% CI 1.1, 4.0)	Age, other pesticides, study site	
	36, 61	Any use OR = 1.6 (95% CI 0.9, 2.8)	Age, other pesticides, study site, priors for chemical class and probability of being carcinogenic [hierarchical model]	
De Roos et al. 2005	71 exposed cases	Any use RR = 1.1 (95% CI 0.7, 1.9)	Age, education, smoking, alcohol, family history, state, 10 pesticides	NHL
(cohort, $n = 57\ 311$)	21 unexposed cases			
	29 cases	1–20 days RR = 1.0 (referent)	same	
	15 cases	21–56 days RR = 0.7 (95% CI 0.4, 1.4)		
	17 cases	57–2678 days RR = 0.9 (95% CI 0.5, 1.6)		
Eriksson et al. 2008	910, 1016 [total]			NHL
(case-control)	29, 18	Any use OR = 2.0 (95% CI 1.1, 3.7)	Age, sex, year of diagnosis or enrollment	
	17, 9	>10 days OR = 2.4 (95% CI 1.0, 5.4)	Same	
Orsi et al. 2009	244, 436 total			NHL
(case-control)	12, 24	Any use OR = 1.0 (95% CI 0.5, 2.2)	Age, center, socioeconomic category	
Cocco et al. 2013	2348, 2462 [total]			B-cell lymphoma
(case-control)	4, 2	Any use OR = 3.1 (95% CI 0.6, 17.1)	Age, sex, education, study center	

CI: confidence interval; HCL: hairy cell leukemia; OR: odds ratio; RR: relative risk.

After removal of duplicates and examining the titles and abstracts, 11 publications were identified as relevant. Reasons for exclusions include: not analytical epidemiology, glyphosate not examined, and NHL and/or MM not examined.

An additional seven relevant analytic epidemiological studies were identified after examining reference lists from the publications above, the IARC Monograph 112 (2015) wherein glyphosate and cancer were evaluated, as well as personal collections of relevant papers by the expert panel. Upon further review, two of these references were excluded: Lee et al. (2005) because it did not focus on NHL or MM (only glioma) and the meta-analysis of Schinasi and Leon (2014) because our focus was on the primary literature. A meta-analysis by Chang and Delzell (2016) that was pending publication at the time of our review would have been excluded for the same reason.

The 16 relevant analytical epidemiological studies are listed in Table 1. Data collected from each study included the following: first author, year of publication, study design, number of cases and controls (for case-control studies), number of participants in cohort studies, results (typically in terms of an estimate of the relative risk [RR], e.g. an odds ratio [OR] with accompanying 95% confidence interval [95% CI]), exposure–response (if available), variables adjusted for in the analyses, and outcome (e.g. NHL, MM). See Tables 2 and 3 for details.

Each study was evaluated by the panel for the following key features that relate to study validity: recall bias (likely/unlikely[3]), exposure misclassification (likely/unlikely), exposure–response analyses with a trend test (yes/no), selection bias (likely/unlikely), adjustment for confounding by other (non-glyphosate) pesticides (yes/no), adjustment for confounding from other variables (yes/no), pathological review of cases (yes/no), proxy respondents (%cases/%controls), bias from sparse data (possible/no), blinding of interviews (yes/no/unclear) and consideration of induction/latency (yes/no). See Table 4 for details.

Validity considerations

Selection bias and recall bias

With the exception of one notable cohort study (De Roos et al. 2005), epidemiologists have employed the case control design to investigate glyphosate. Case control and cohort studies are related designs. Both study designs, if conducted with high quality, can produce valid results. In fact, the case control design is best thought of as including the cases that would have been detected in a hypothetical cohort study

Table 3. Results for glyphosate: multiple myeloma (MM).

Author, year (study design)	# cases, controls Total or exposed	OR/RR (95% CI)	Multivariate adjustments	Outcome
Brown et al. 1993	173, 650 [total]			
(case-control)	11, 40	Any use OR = 1.7 (95% CI 0.8, 3.6)	Age, vital status	MM
De Roos et al. 2005	24 exposed cases	Any use RR = 1.1 (95% CI 0.5, 2.4)	Age	MM
(cohort, $n = 57\ 311$)	Eight unexposed cases			
	Not specified	Any use RR = 2.6 (95% CI 0.7, 9.4)	Age, education, smoking, alcohol, family history, state, 10 pesticides	
	Eight exposed cases	1–20 days RR = 1.0 (referent)	Age, education, smoking, alcohol, family history, state, 10 pesticides	
	Five exposed cases	21–56 days RR = 1.1 (95% CI 0.4, 3.5)		
	Six exposed cases	57–2678 days RR = 1.9 (95% CI 0.6, 6.3)		
Orsi et al. 2009	56, 313 [total]			MM
(case-control)	5, 18	Any use OR = 2.4 (95% CI 0.8, 7.3)	Age, center, socioeconomic category	
Kachuri et al. 2013	342, 1357 [total]			MM
(case-control)	23, 108	Any use OR = 1.1 (95% CI 0.7, 1.9)	Age, province, smoking, selected medical conditions, family history of cancer	
			Same	
	11, 78	≤2 days/year OR = 0.7 (95% CI 0.4, 1.4)		
	10, 26	>2 days/year OR = 2.1 (95% CI 0.95, 4.7)		
Sorahan 2015	24 exposed cases	Any use RR = 1.1 (95% CI 0.5, 2.5)	Age	MM
	Eight unexposed cases			
Reanalysis of De Roos et al. 2005				
	24 exposed cases	Any use RR = 1.2 (95% CI 0.5, 2.9)	Age, sex, education, smoking, alcohol, family history of cancer, education, 10 pesticides	
	Eight unexposed cases			
	Eight cases	Never used RR = 1.0 (referent)	Age, sex, education, smoking, alcohol, family history of cancer, education, 10 pesticides	
	10 exposed cases	1–20 days RR = 1.1 (95% CI 0.4, 3.0)		
	Eight exposed cases	21–57 days RR = 1.5 (95% CI 0.5, 4.3)		
	Six exposed cases	57–2678 days RR = 1.4 (95% CI 0.4, 4.5)		

CI: confidence interval; HCL: hairy cell leukemia; OR: odds ratio; RR: relative risk.

1. Reanalysis of De Roos et al. to assess the exclusion of 14 000 with some missing covariate data as the explanation for the difference in RRs adjusted for age (RR = 1.1) versus adjusted for age, education, smoking alcohol, family history, state and 10 pesticides (OR = 2.6).

along with a sample of the source population (Rothman et al. 2008). The purpose of the control group is to determine the relative size of the exposed and unexposed populations that gave rise to the cases, so as to enable valid risk estimates for exposed versus unexposed populations. At times in case control studies, the control population is selected for convenience or practicality in a way that does not allow determining the relative size of the exposed and unexposed populations. For example, hospital controls may be less likely to have strenuous occupations than the general population; hence farmers and/or others with pesticide exposures might be under-represented among hospital controls. Poor or selective participation by potential controls can produce the same result. Both scenarios are examples of selection bias that would almost certainly generate spurious positive associations between farming exposures and cancers.

A particularly important and well-known potential bias in case control studies of pesticides is recall bias. That is, cases tend to be more likely to remember or report exposures than are study participants who have not been diagnosed with cancer. This bias results from the natural self-examination by cases of what might have caused their grievous illness. Recall bias is not a concern in the sole glyphosate cohort study (De Roos et al. 2005) because exposure was determined from study participants at study entry before follow-up began for health outcomes. Recall bias tends to produce spurious positive associations between exposure and disease.

Concern about recall bias also extends to next-of-kin who participate in epidemiologic studies in place of deceased or disabled family members. Analyses of next-of-kin or proxy respondents have been found to produce results similar to those of first-hand study subjects (e.g. Kachuri et al. 2013) or to show results quite different than those based on first-hand responders (e.g. Lee et al. 2005 – ORs for glyphosate and glioma were 0.4 based on primary respondents and 3.1 for proxy respondents); one never knows the impact of having appreciable numbers of next-of-kin respondents without a thorough analysis of data with/without proxy respondents (Johnson et al. 1993). This concern is noteworthy because the case-control studies for glyphosate frequently have a high proportion of next-of-kin participants and many studies did not evaluate the potential bias from next-of-kin responders.

Table 4. Validity considerations for glyphosate studies.

First author, year	Recall bias	Exposure misclassification	Exposure–response and trend test	Selection bias	Adjusted for confounding from other pesticides yes/no	Adjusted for confounding from other variables yes/no	Pathology review of cases	Proxies %cases/%controls	Bias from sparse data	Blinding of interviews	Consideration of latency
Brown et al. 1993	Likely	Moderate ever/never	No	Unlikely	No	Yes	Yes	42% for cases; 30% for controls	No	Unclear	No
McDuffie et al. 2001	Likely	Moderate ever/never; appreciable days of use	Yes, no trend test	Likely	No	Yes and no	Yes	21% cases; 15% controls	No	Unclear	No
Hardell et al. 2002	Likely	Moderate ever/never; appreciable in days of use analysis	No	Unlikely	Yes, but variables not specified	Unclear	Yes for NHL, unclear for HCL	43% NHL cases and controls, 0% for HCL	Possible	Yes	No
De Roos et al. 2003	Likely in original publications	Moderate ever/never	No	Likely, in original publications	Yes	Yes	Yes	31% for cases; 40% for controls	No	Yes	No
De Roos et al. 2005	No	Moderate ever/never; appreciable in days of use analysis	Yes, yes	Unlikely	Yes	Yes	Yes	No	Possible in some analyses	N/A	No
Eriksson et al. 2008	Likely	Moderate ever/never	Yes, no trend test	Unlikely	Yes	Age, sex, year of diagnosis	Yes	No	Possible in some analyses	Yes	Yes
Orsi et al. 2009	Likely	Moderate ever/never	No	Likely	No	Yes	Yes	No	Possible	Yes	No
Cocco et al. 2013	Likely	Likely	No	Likely	No	No	20%	No	Possible	Unclear	No
Kachuri et al. 2013	Likely	Moderate ever/never; appreciable in days of use analysis	Yes, no trend test	Likely	No	Yes	Yes	Excluded	No	Unclear	No

NHL: non-Hodgkin's lymphoma.

Exposure assessment and misclassification

With few exceptions, epidemiologic studies of pesticides assess exposure by questioning participants or their next-of-kin about the prior use of specific pesticides and associated work practices. This practice has limitations compared with other branches of occupational research where epidemiologists often have access to objective documentation about past industrial workplace conditions to aid in exposure assessment (e.g. engineering diagrams, process descriptions, job descriptions, area or personal exposure-monitoring data).

A number of publications provide insights about the validity or reliability of self-reported pesticide information used in epidemiologic studies. In one study, approximately 60% of farmers' self-reports agreed with suppliers' records of purchases for specific pesticides (Hoar et al. 1986). In another article, researchers evaluated the repeatability of self-reported pesticide information on enrollment questionnaires for 4188 licensed pesticide applicators, primarily farmers, who filled out questionnaires in successive years (Blair et al. 2002). The year-to-year reliability for reporting any lifetime use of 11 widely used pesticides varied from 79 to 87%; categorical agreement varied from 50 to 59% for typical days of use per year and from 50 to 77% for years of use. Based on this literature, it is apparent that perhaps 10–20% or more of participants in epidemiologic studies may report incorrectly that they have used a specific pesticide and that reporting on frequency of use and years of use is even less certain.

There seems to be considerable under-appreciation of the implications of the acknowledged degree of exposure misclassification in the pesticide literature. Many consider exposure misclassification to almost always be non-differential (e.g. similar for cases and controls) and, therefore, to bias analyses toward the null (or no association between an exposure and a disease). However, even assuming the misclassification is non-differential overall over multiple analyses, the direction of the resulting bias can be uncertain for any specific analysis. As Rothman and Greenland (1998) pointed out, in any given study, random fluctuations can lead to bias away from the null (towards a positive or negative association) even if the classification method satisfies all the conditions for being non-differential (viz. on average). Hence, in the studies considered in this review, with hundreds of comparisons per study, some fraction of results likely will be biased away from the null even if misclassification is non-differential.

Finally, unlike the five days per week, 50 weeks per year routine for exposures in industrial settings, glyphosate and other pesticide applications are not a frequent occurrence for farmers and applicators. In fact, for most, application of a specific pesticide, like glyphosate, is seasonal and happens only a few days per year. The high exposure category in the glyphosate literature is usually two or more days per year – reflecting extremely infrequent use for the great majority of study subjects and, annually, long periods without exposure. This implies that pesticide exposures are much less frequent than other occupational exposures for those who use pesticides in their occupations and that these other, daily exposures need to be addressed comprehensively in any analysis of infrequently used pesticides.

Biomonitoring studies, implications for exposure assessment

Epidemiologists recognize that there is a difference between exposure (viz. reported use) and dose (the quantity of a substance that is absorbed). In fact, dose is of more interest than exposure in studying potential causal associations. For some chemicals, exposure and dose correlate well. For other chemicals, the correlation is low. Understanding the correlation between exposure and dose is essential for exposure–response analyses – an important indicator for a causal relationship.

The properties of a chemical affect dose. Glyphosate is usually formulated as the isopropylamine salt, which has an extremely low vapor pressure of 1.6×10^{-8} mm Hg (Tomlin 2003). Inhalation of spray droplets was found to be a minor route of glyphosate exposure in a study of glyphosate applicators in Finland (Jauhiainen et al. 1991), leaving dermal contact as the primary route of exposure. Dermal penetration experiments, where glyphosate was left undisturbed on skin surfaces of experimental animals and on human skin *in vitro*, indicate a percutaneous absorption of less than 2% (Wester et al. 1991).

Biomonitoring studies show results consistent with glyphosate's physical/chemical properties. In a study of 48 farmers in Minnesota and South Carolina during a normal day of glyphosate application on their farms, 60% of applicators were found to have quantifiable glyphosate in urine (the predominant route of excretion), while 40% of farmers did not (Acquavella et al. 2004). The distribution of urinary concentrations was highly skewed, with only a small percentage of values appreciably different than the one part per billion limit of detection. Nine farmers completed applications in excess of 100 acres and did not have detectable values for glyphosate in their urine. Evaluation of different approaches to exposure assessment used in epidemiologic studies has not shown good correlation with biomonitoring data for glyphosate (Acquavella et al. 2006), implying appreciable misclassification in studies that rely on traditional pesticide exposure assessment approaches.

The maximum systemic dose found in a review of all glyphosate biomonitoring studies completed to date is 0.004 mg/kg (Niemann et al. 2015). For comparison, the US Environmental Protection Agency (US EPA)'s reference dose (viz. the daily oral exposure to the human population, including sensitive subgroups such as children, that is not likely to cause harmful effects during a lifetime) is 500-fold higher at 2 mg/kg/day (US EPA 1993). The geometric mean systemic glyphosate dose for applicators is 0.0001 mg/kg/day.

Statistical considerations

In addition to the potential study biases discussed above, other threats to validity arise from the statistical procedures used (or not used) in the epidemiology studies reviewed for glyphosate. First, glyphosate risk estimates in several studies were based on small numbers of events in the exposure subcategories considered. For example, the case-control studies of NHL reported by Hardell et al. (2002), Cocco et al. (2013), and Eriksson et al. (2008) and of MM reported by Orsi et al. (2009) involved less than 10 exposed cases and/or controls

overall or in specific glyphosate exposure categories. Even the large cohort study of 57 311 pesticide applicators conducted by De Roos et al. (2005) and reanalyzed by Sorahan (2015) included sparse data (viz., 10 or fewer glyphosate-exposed MM cases in each of the three exposure categories considered).

Sparse data not only leads to imprecise risk estimates, but can decrease their validity when analyses are limited to asymptotic procedures (Greenland et al. 2000; Hirji 2006). The phenomenon of a bias away from the null due to small samples or sparse data is termed sparse data bias. It can occur if case-control or cohort studies are analyzed by conventional asymptotic methods such as logistic regression or Poisson regression rather than their counterparts based on exact estimation. For example, in the presence of sparse data, the estimated OR derived from asymptotic conditional logistic regression is substantially overestimated if the true OR is greater than one (Breslow & Day 1980). Sparse data bias also affects estimated CIs and *p* values (Greenland et al. 2000; Subbiah & Srinivasan 2008). It appears that all studies involving sparse data relied upon asymptotic procedures only, and were thus likely subject to sparse data bias and inflated risk estimates.

As shown in Table 4, with few exceptions, the statistical models used to evaluate NHL or MM risks among pesticide-exposed individuals were deficient at many levels. As all studies were exploratory (viz. not testing *a priori* hypotheses regarding specific pesticide exposures and NHL or MM risk), they produced a large number of risk estimates along with a high probability of some estimates being statistically significant simply due to chance alone. No attempt was made in any of the studies to adjust *p* values for these multiple comparisons, though one case control study (De Roos et al. 2003) used a two stage hierarchical modeling approach to adjust risk estimates based on pesticide class characteristics and extant carcinogenic classification to minimize false positives. Also, as shown in Table 4, most studies did not adjust glyphosate risk estimates for potential confounding by other pesticide exposures or relevant medical variables, and only one (Eriksson et al. 2008) considered latency period or the time between first (or last) glyphosate exposure and health outcome. Moreover, only one study (Hohenadel et al. 2011), considered the possible interaction or effect modification between pairs of commonly used pesticides.

Even among the few studies that incorporated potential confounding or effect modifying factors, little if any information was provided about the statistical model selection (e.g. asymptotic or exact), model building strategy (e.g. criteria for including/excluding co-variables) or the diagnostic procedures used to evaluate the fit or robustness of intermediate and final models. Thus, in most studies, reported glyphosate risk estimates remained relatively crude (viz. not fully adjusted) and likely biased due to residual confounding, poor model fit and in some cases, sparse data.

NHL studies

Cantor et al. (1992) conducted a NHL case control study in Iowa and Minnesota to evaluate possible causal factors, including pesticides. The data from this study were pooled with two other US NHL case control studies and subsequently reported by De Roos et al. (2003). We defer consideration to that more recent analysis.

Nordstrom et al. (1998) conducted a population-based case control study in Sweden that included 121 cases of hairy cell leukemia (HCL) and 484 general population controls. The intent of the study was to evaluate occupational exposures and smoking as risk factors for HCL. The data from this study are included with data from the Hardell and Eriksson (1999) study in a later publication (Hardell et al. 2002). We defer consideration of both primary studies to that more recent analysis.

McDuffie et al. (2001) conducted a trans-Canada multi-center case control study to evaluate the relationship between pesticide exposures and NHL. Cases ($n = 517$) were identified from provincial Cancer Registries except in Quebec, for which hospital ascertainment was used. Controls ($n = 1506$) were selected at random from the provincial Health Insurance records (Alberta, Saskatchewan, Manitoba, Quebec), computerized telephone listings (Ontario) or voters' lists (British Columbia). Participation was much higher among invited cases (67%) than among invited controls (48%). Pesticide exposure was determined through telephone interviews of study participants or their proxies (21% of cases, 15% of controls). The authors used conditional logistic regression to estimate ORs. The OR for any reported glyphosate use was 1.2 (95% CI 0.8–1.7) controlling for age, province and medical variables associated with NHL. The strongest pesticide associations were with mecoprop (OR = 2.3) and dicamba (OR = 1.9). A subsequent analysis by reported days of use per year (none, $\leq$2 days/year, $>$2 days/year) showed glyphosate ORs of 1.0, 1.0 (95% CI 0.6–1.6), and 2.1 (95% CI 1.3–2.7), respectively. This latter analysis did not adjust for medical variables that were controlled in the analysis of any glyphosate use or for the effects of other pesticides.

Assessment: The strengths of this study are the relatively large number of NHL cases and the likelihood that almost all cases were confirmed histologically. The limitations are likely residual confounding in the analysis by days of use by the uncontrolled effects of medical variables and other pesticides, selection bias (differential participation by cases and more proxies for cases), and possible recall bias.

Hardell et al. (2002) reported a pooled analysis of two case control studies; one of NHL and the other of HCL. Both of these studies were previously reported as separate case-control studies (Nordstrom et al. 1998; Hardell & Eriksson 1999). HCL is rare, comprising 2% of lymphoid leukemias, and typically affects middle aged to elderly men (Foucar et al. 2008). It is regarded as a mature B cell neoplasm, as are a high proportion of NHLs. It appears that the authors pooled the two separate studies principally to achieve a larger study size under the assumption that the two neoplasms could be treated as a homogeneous entity for etiologic research. However, the pooled analysis is thereby heavily weighted by HCL cases and the results not representative of NHL more broadly. The 404 NHL cases were males aged 25 and older, diagnosed in 1987–1990, and living in mid- and northern Sweden, drawn from regional cancer registries (viz.

histologically verified). Each case was matched on age and sex to two controls drawn from the National Population Registry. The 111 HCL cases were males diagnosed in 1987–1990, identified from the Swedish Cancer Registry covering the whole country. Each HCL case was matched on age, sex and county to four controls drawn from the National Population Registry. A total of 515 cases and 1141 controls were included in pooled analyses of NHL and HCL. A questionnaire was completed by study subjects or next-of-kin regarding complete working history and exposure to various chemicals. Exposure to each chemical was dichotomized, with at least one working day a year before diagnosis being regarded as positive for exposure. Conditional logistic regression was used to estimate ORs and 95% CIs, adjusted for study (NHL versus HCL), study area, and vital status. In the analyses, only subjects with no pesticide exposure were regarded as unexposed[4], whereas subjects who had not used glyphosate but had used other pesticides were excluded. Analysis for glyphosate, unadjusted for other pesticides, showed a positive association (OR = 3.0, 95% CI 1.1–8.5) based on eight exposed cases and eight exposed controls. Although multivariate analyses were done, it was not stated how variables were selected for inclusion or which variables were included in the multivariate models. The multivariate model for glyphosate indicated appreciable confounding in the unadjusted analysis and a reduced, statistically imprecise, positive association for glyphosate (OR = 1.9, 95% CI 0.6–6.2). Analyses based on increasing days of use were presented for some pesticides, but not for glyphosate.

Assessment: The strengths of this study were that cases were histologically confirmed and controls were population-based. The limitations of this publication were many. First, the investigators found a positive association for every class of pesticide and for every individual pesticide, suggesting a systematic bias in either the assessment of exposure (e.g. recall bias, interviewer or subject (inadvertent) unblinding), in the reporting of results, or due to selection bias. Second, the definition of unexposed (viz. no exposure to any pesticide) used in the analysis distorted the exposure prevalence for glyphosate and precluded being able to control for possible confounding by other pesticides and farming exposures. Third, there seems to be some inconsistency in exposure assessment between the two studies that were pooled in this publication. The prevalence of exposure to glyphosate was three times higher among HCL cases and controls (1.3%) than it was among NHL study subjects (0.4%), even though both studies were contemporaneous and would be expected to have similar exposure prevalences.

De Roos et al. (2003) reported a pooled analysis of three NHL case-control studies of pesticides and other potential causal factors (Hoar et al. 1986; Zahm et al. 1990; Cantor et al. 1992). This analysis was limited to men and excluded cases and controls with a history of living or working on a farm before (but not after) age 18. Cases from the Nebraska study by Zahm et al. (1990) were diagnosed between July 1983 and June 1986 and were identified using the Nebraska Lymphoma Study Group as well as data from area hospitals. Cases from the Kansas study by Hoar et al. (1986) represented a random sample of cases diagnosed between 1979 and 1981 and selected from the Kansas Cancer Data Service. Cases from the study in Iowa and Minnesota by Cantor et al. (1992) were diagnosed between 1981 and 1983 and were identified from the Iowa State Health Registry along with a surveillance system established in Minnesota. Controls for these studies were randomly selected from population databases (e.g. Medicare, random digit dialing, and state mortality files for deceased cases) and frequency matched to cases on race, sex, age and vital status at time of interview. Cases and controls were interviewed (including next-of-kin when necessary) regarding use of pesticides and/or herbicides as well as other known or suspected risk factors for NHL. The final analysis dataset included 650 cases and 1933 controls, after exclusions of individuals for whom there was missing information. Forty-seven pesticides were included in the analysis after excluding pesticides for which there were not at least 20 persons exposed and data available from all three studies. The exposure metric in the analysis was restricted to any reported use of a specific pesticide, with no consideration of extent of use. Two types of statistical models were used to estimate ORs and 95% CIs: (1) standard logistic regression and (2) hierarchical regression, wherein logistic regression estimates were adjusted in a second stage based on expected similarities of effects within pesticide classes and the presumed *a priori* carcinogenic probability for specific pesticides as determined by external review bodies. For pesticides like glyphosate that were presumed to have a low probability of being carcinogenic, this second stage adjustment tended to draw positive associations toward the null. All analyses were adjusted for age and for the use of 46 other pesticides. Results for glyphosate showed an OR of 2.1 (95% CI: 1.1–4.0) in the logistic regression and a lesser association (OR = 1.6, 95% CI: 0.9–2.8) in the hierarchical regression.

Assessment: The strengths of this analysis were the histological confirmation of NHL cases and the large numbers of cases and controls that enabled simultaneous adjustment of the effects of 47 pesticides. The weaknesses of this study were the reliance on a relatively crude indicator of exposure (ever having used a pesticide with no consideration of the extent of use) and the limitations common to case control studies of pesticides – namely recall bias and, in this case, an appreciably higher proportion of proxy respondents for controls than cases (40% versus 31%).

De Roos et al. (2005) reported glyphosate findings from the Agricultural Health Study (AHS), a large prospective cohort study of health outcomes related to numerous pesticides among more than 53 000 licensed pesticide applicators in North Carolina and Iowa. Analyses for glyphosate considered potential exposure in a number of ways including: ever/never use, estimated cumulative exposure days (CED), and estimated intensity-weighted exposure days (IWED). The statistical approach was Poisson regression and effects were estimated as RRs with 95% CIs. After adjusting for age, findings for ever/never use of glyphosate showed a near null RR of 1.2 for NHL (95% CI 0.7–1.9), based on 92 cases. Further adjustment for education level, pack-years of smoking, alcohol use in last 12 months, family history of cancer, state of residence and 10 other pesticides that were correlated with glyphosate use, and excluding applicators who had missing data for any of these variables, had little effect on findings for NHL (RR 1.1 95% CI 0.7–1.9). Analyses of potential exposure–response effects using the first tertile of CEDs as a baseline category and with adjustments as described above, and

excluding the never-users from the analysis, found a slight non-significant negative trend (1–20 days: RR 1.0; 21–56 days: RR 0.7, 95% CI 0.4–1.4; 57–2678 days: RR 0.9, 95% CI 0.5–1.6). These categorical analyses were repeated for IWEDs and findings were little changed. De Roos et al. (2005) qualified their results as being based on small numbers, but concluded: "… the available data provided evidence of no association between glyphosate exposure and NHL incidence."

Assessment: The strengths of this study are the large size of the study cohort, the high quality assessment of cancer incidence based on statewide registries in Iowa and North Carolina, the lack of proxy respondents, the control for confounding by other pesticides, and the fact that collection of information about pesticide use could not be influenced by health status. The limitations of the study are the relatively short duration of follow-up for AHS cohort members, the relatively small number of NHL cases, and the likelihood of some degree of exposure misclassification in the various analyses.

Eriksson et al. (2008) reported a population based case control study of NHL in males and females aged 18–74 living in Sweden in 1999–2002. Cases were identified through physicians who diagnosed and treated NHL, and all cases were histologically verified. Controls were randomly chosen from population registries in the same health service regions as the cases, and were frequency matched in 10-year age and sex groups. A total of 910 NHL cases and 1016 controls were included in the analyses. The authors emphasized that, in contrast to their previous studies (Hardell et al. 1981; Hardell & Eriksson 1999), the analyses evaluated newer types of pesticides in relation to different histopathological subtypes of NHL. All subjects received a mailed questionnaire focusing on total work history and exposure to pesticides, solvents and other chemicals. For all pesticides, the number of years, number of days per year and length of exposure per day were questioned. Exposure to each chemical was dichotomized, with at least one working day at least a year before diagnosis being regarded as positive. In the analyses, only subjects with no pesticide exposure were regarded as unexposed[5], whereas subjects with other pesticide exposures were excluded. Unconditional logistic regression was used to calculate ORs and 95% CIs, adjusted for age, sex, and year of diagnosis. Analyses for individual herbicides showed positive associations for every agent and ORs were elevated for every other pesticide (although not in every analysis by NHL subtype or category of duration of exposure). In the model for glyphosate and all NHL (not adjusted for other exposures), the OR was 2.0, 95% CI 1.1–3.7 for ever/never exposure, based on 29 exposed cases and 18 exposed controls. Exposure to glyphosate for >10 days showed OR = 2.4, 95% CI 1.0–5.4 (not adjusted for other exposures). Analyses of glyphosate exposure and NHL subtypes (not adjusted for other exposures) were positive for every subtype of NHL, and were statistically significant for lymphocytic lymphoma/B-CLL (OR = 3.4, 95% CI 1.4–7.9) and unspecified NHL (OR = 5.6, 95% CI 1.4–22.0). Results for other NHL subtypes were not statistically significant: all B-cell NHL (OR = 1.9, 95% CI 0.998–3.5); follicular NHL (OR = 1.9, 95% CI 0.6–5.8); DLBCL (OR = 1.2, 95% CI 0.4–3.4); other B-cell NHL (OR = 1.6, 95% CI 0.5–5.0); unspecified B-cell NHL (OR = 1.5, 95% CI 0.3–6.6) and T-cell NHL (OR = 2.3, 95% CI 0.5–10.4). Multivariate analysis of glyphosate exposure was stated to include agents with statistically significant increased ORs or with an OR >1.5 and at least 10 exposed subjects. These models excluded subjects with exposure to pesticides that did not meet these conditions. The multivariate model for glyphosate and all NHL showed a non-significant positive association (OR = 1.5, 95% CI 0.8–2.9) for ever/never exposure, indicating substantial confounding in the analysis that were not adjusted for other pesticides.

Assessment: Strengths of the study include histological verification of cases and use of population-based controls. There were, however, a couple of major limitations. First, the investigators found a positive association for every herbicide and for every individual pesticide (although not in every sub-analysis), suggesting a systematic bias in either the assessment of exposure (e.g. recall bias, interviewer or subject [inadvertent] unblinding), in the reporting of results, or due to selection bias. Second, the definition of unexposed (viz. no exposure to any pesticide) used in the analysis distorted the exposure prevalence for glyphosate for cases and controls and precluded being able to control for possible confounding by other pesticides and farming exposures.

Hohenadel et al. (2011) conducted a reanalysis of data included in the McDuffie publication to evaluate the relationship between exposure to specific pesticide combinations and NHL. The authors used unconditional logistic regression to estimate ORs for the total number of pesticides used by type and carcinogenic potential and for pairwise pesticide combinations (neither, either only or both). Where the OR for joint exposure was higher than the OR for exposure to either pesticide alone, interaction on the additive scale was evaluated using an interaction contrast ratio (ICR). Exposure to glyphosate alone yielded an estimated 8% deficit in NHL risk (OR = 0.92, 95% CI 0.5–1.6), whereas use of malathion only was associated with an elevated NHL risk (OR = 2.0, 95% CI 1.3–2.9). The OR of 2.1 (95% CI 1.3–3.4) for joint exposure to glyphosate and malathion was similar to that for malathion alone and there was no indication of a super additive joint effect (ICR <0.5).

Assessment: The strengths and limitations of this study are similar to those outlined for the related study by McDuffie et al. (2001). The re-analysis was more an exploratory assessment of joint exposures than it was a study of specific pesticides *per se* and is of limited relevance for a possible association between glyphosate and risk of NHL.

Orsi et al. (2009) reported a hospital-based case-control study of occupational exposure to pesticides and lymphoid neoplasms (including but not limited to NHL and MM) undertaken in France. Incident cases of NHL ($n = 244$) were identified from six French hospital center catchment areas between 2000 and 2004. A panel of pathologists and hematologists confirmed pathology. Controls ($n = 436$) were selected from the same hospitals as cases; controls had no history of lymphoid neoplasms and were primarily patients from rheumatology and orthopedic departments. Patients admitted for occupation-related diseases or diseases related to smoking and/or alcohol abuse were not eligible as controls although a past history of such diseases/conditions did not eliminate the control. Controls were matched to cases by center, age (±3 years) and gender. Information on cases and controls

involved a standardized self-administered questionnaire on socioeconomic status, family medical history, and lifelong residential and occupational histories. For additional information (on personal and family history), smoking, alcohol, tea and coffee consumption, use of pesticides (insecticides, fungicides, and herbicides) as well as detailed questions about work on farms, a trained interviewer performed a face-to-face interview with cases and controls. Two exposure definitions were used: definite or possible. Duration of exposure was estimated. ORs and 95% CIs were calculated using logistic regression. Results for any use of glyphosate and NHL showed no association (OR = 1.0, 95% CI: 0.5–2.2) based on 12 exposed cases and 24 exposed controls.

Assessment: A strength of this study is that the NHL cases were confirmed histologically. The limitations are no assessment of potential confounding due to the uncontrolled effects of other pesticides/exposures, possible recall bias and selection bias (controls were primarily selected from orthopedic and rheumatological departments where general population prevalence of pesticide exposure would likely be under-represented). Scanning the ensemble of hundreds of effect estimates shows that the vast majority of estimates (though not for glyphosate) were greater than one, suggesting systematic error across the various analyses.

Cocco et al. (2013) reported results from the EPILYMPH case control study of NHL in six European countries, conducted in 1998–2004. The study included 2348 incident lymphoma cases and 2462 controls. Approximately 20% of the cases had their tissue slides reviewed by a central panel of pathologists. Controls were population-based in Germany and Italy, matched on gender, age (within five years) and residence area. Hospital controls were used in the Czech Republic, France, Ireland and Spain, excluding patients with diagnoses of cancer, infectious disease, and immunodeficiency. The participation rate was 88% in cases, 81% in hospital controls, but only 52% in population controls in Germany and Italy (Cocco et al. 2010). Trained interviewers conducted in-person interviews with a structured questionnaire regarding full time jobs held for a year or longer. Industrial hygienists coded the occupations to the ISCO, International Labour Office (1968) and the NACE, Statistical Office of the European Communities (1996) classifications. Subjects who reported having worked in agriculture were given a job-specific module inquiring in detail about tasks, kinds of crops, size of cultivated area, pests being treated, pesticides used, procedures of crop treatment, use of personal protective equipment, reentry after application and frequency of treatment in days/year. Hygienists reviewed the job modules to assess exposure to pesticides in categories. Exposure was scored in terms of confidence (probability and proportion of workers exposed), intensity and frequency. A cumulative exposure score was calculated. Subjects unexposed to any pesticide[6] were the referent category for all analyses. Unconditional logistic regression was used to calculate ORs and 95% CIs, adjusted for age, gender, education and study center. The authors reported a moderate association between glyphosate (ever/never exposure) and B-cell NHL (OR = 3.1, 95% CI 0.6–17.1) in a univariate analysis that was statistically imprecise being based on only four exposed cases and two exposed controls. Clearly, there were too few exposed cases and controls to estimate an OR for glyphosate controlling for other exposures.

Assessment: Glyphosate exposure was so infrequent in this study that it precluded an informative analysis. Were that not the case, there would have been obvious concerns about selection bias (esp. low participation for controls), confounding by other exposures (esp. solvent exposures found to be associated with NHL is a previous analysis of this data (Cocco et al. 2010), and recall bias. In addition, the definition of unexposed (viz. no exposure to any pesticide) used in the analysis distorted the exposure prevalence for glyphosate and would have precluded being able to control for possible confounding by other pesticides and farming exposures had such analyses been attempted.

MM studies

Brown et al. (1993) conducted a re-analysis of the National Cancer Institute Iowa population-based case-control study (Brown et al. 1990; Cantor et al. 1992) to evaluate the relationship between exposure to specific pesticides and MM. Cases ($n = 173$) were identified from the Iowa Health Registry. Controls ($n = 650$) were frequency matched to cases by age group and vital status at interview and selected from three sources: random digit dialing (living cases under age 65); Medicare records (living cases aged 65+) and state death certificate files (for deceased cases). Participation was relatively high and similar among cases (84%) and controls (78%). Pesticide exposure for 34 crop insecticides, 38 herbicides (including glyphosate) and 16 fungicides was determined from in-person interviews with subjects or their proxies. The authors used unconditional logistic regression to estimate ORs for pesticides handled by at least five cases. Subjects who did not farm[7] were the referent exposure category for these analyses. The OR for mixing, handling or applying glyphosate was 1.7 (95% CI 0.8–3.6) adjusted for vital status and age. Failure to use protective equipment (obtained from interviews) did not appreciably increase the risk for glyphosate (OR = 1.9, 95% CI not reported). None of the pesticides considered showed a statistically significant association with MM risk.

Assessment: Strengths of the study were the histological confirmation of cases and the high and similar participation for cases and controls. Study limitations were its exploratory nature (as noted by the authors), lack of control for potential confounding by possibly relevant personal characteristics or by exposure to other pesticides, and possible recall bias. In addition, the definition of unexposed (viz. non-farmers) used in the analysis excluded 64% of cases and 58% of controls, distorted the exposure prevalence for glyphosate, and would have precluded being able to control for possible confounding by other pesticides and farming exposures had the investigators sought to control potential confounding.

De Roos et al. (2005), based on data from the AHS cohort study described previously, estimated the age-adjusted RR for glyphosate and MM to be 1.1 (95% CI 0.5–2.4), based on 32 cases. Further adjustment for education level, pack-years of smoking, alcohol use in the last 12 months, family history of cancer and state of residence, together with the use of 10 other pesticides that were correlated with glyphosate use, and excluding approximately 14 000 applicators and 13 MM cases with missing data for any of these variables, markedly

increased the RR for MM (RR = 2.6, 95% CI 0.7–9.4). Analyses of exposure–response effects using the first tertile of CEDs as a baseline category and with adjustments as described above, and excluding the never-users from the analysis, produced a non-significant positive trend (1–20 days: RR = 1.0; 21–56 days: RR = 1.1, 95% CI 0.4–3.5; 57–2678 days: RR = 1.9, 95% CI 0.6–6.3; *p* values for trend 0.27). This MM CED analysis was based on 19 (of 32) cases, the other 41% of cases being excluded for any missing covariate information. These analyses were repeated for IWED categories and findings were little changed (RRs 1.0, 1.2, and 2.1; *p* values for trend = 0.17). The authors also repeated the exposure–response analyses for MM, using the never-use group as the baseline category and found a monotonic positive trend (tertile 1: RR = 2.3; 95% CI 0.6–8.9; tertile 2: RR = 2.6; 95% CI, 0.6–11.5; tertile 3: RR = 4.4; 95% CI 1.0–20.2; *p* values for trend = 0.09). The authors noted that the marked difference between the age adjusted MM findings and the more fully adjusted findings (viz. RR = 1.1 versus 2.6) could have been due to selection bias related to the 14 000 AHS cohort members who were dropped from the more fully adjusted analysis due to missing values for one or more variables.

Assessment: The strengths of this study are the large size of the study cohort, the high quality assessment of cancer incidence based on statewide registries in Iowa and North Carolina, the lack of proxy respondents, the control for confounding by other pesticides, and the fact that collection of information about pesticide use could not be influenced by health status. The limitations of the study are the short duration of follow-up for AHS cohort members, the relatively small number of MM cases, the likelihood of some degree of exposure misclassification in the various analyses, and the indications of selection bias affecting RR estimates due to the exclusion of so many cohort members and MM cases from the more fully adjusted analyses (addressed in a subsequent publication by Sorahan 2015).

Orsi et al. (2009) reported a French hospital-based case-control study of occupational exposure to pesticides and lymphoid neoplasms (including but not limited to NHL and MM), described previously. Included were 56 incident cases of MM and 313 controls matched to cases by center, age (±3 years) and gender. ORs and 95% CIs were calculated using logistic regression. Results for glyphosate and MM showed a moderate, but statistically imprecise, association (OR = 2.4, 95% CI: 0.8–7.3) based on five exposed cases and 18 exposed controls.

Assessment: A strength of this study is that the MM cases were confirmed histologically. The limitations are likely residual confounding due to the uncontrolled effects of other pesticides/exposures in the assessment of the OR for glyphosate, possible recall bias, and selection bias (controls were primarily selected from orthopedic and rheumatological departments where general population prevalence of pesticide exposure would likely be under-represented). Scanning the ensemble of hundreds of ORs shows that the vast majority was greater than 1.0, suggesting systematic error across the various analyses.

Landgren et al. (2009) estimated the age-specific prevalence of monoclonal gammopathy of undetermined significance (MGUS) (a medical condition that is sometimes a precursor to multiple myeloma) among a stratified random sample of 678 AHS participants selected based on lifetime organophosphate use. Subjects in the sample had completed all three phases of the AHS questionnaires, were enrolled into a neurobehavioral study nested within the AHS cohort, and had provided serum for analysis. The authors compared MGUS prevalence for this sample to that for the general population of Olmsted County, Minnesota (due to availability of Mayo Clinic MGUS screening data) and found higher prevalence for AHS participants. Within the AHS sample, associations between MGUS prevalence and pesticide exposures and subject characteristics were assessed in logistic regression models adjusted for age and education level. The prevalence OR for MGUS for glyphosate users versus non-users, adjusted for age and education level, was 0.5 (95% CI 0.2–1.0). None of the herbicides studied showed a strong association with MGUS.

Assessment: This is a small exploratory study of pesticide effects on a medical condition that is sometimes a precursor to MM. Taken at face value, the results provide evidence of a weak inverse association between risk of MGUS and glyphosate, though the exploratory nature of this study, the lack of adjustment for other pesticides in pesticide-specific analyses, the cross-sectional nature of the study, and the implied speculative hypothesis underlying the analysis (that pesticides might cause MM by causing MGUS first) limit conclusions that can be drawn from this work.

Pahwa et al. (2012) reported a trans-Canada, multi-center case control study regarding the relationship between pesticide exposures and MM. The publication is related to the trans-Canada NHL study reported initially by McDuffie et al. (2001) wherein there was a common control group for the study of several lymphopoietic cancers. Pahwa et al. (2012) was updated by Kachuri et al. (2013) and we defer consideration to that more recent publication.

Kachuri et al. (2013) presented a reanalysis and extension of Pahwa et al. (2012) in which they excluded 149 (of 1506) controls who did not have an age match with the MM cases. Kachuri et al. utilized unconditional logistic regression to estimate ORs and presented analyses including and excluding proxy respondents (15% of controls and 30% of cases) and adjusting for smoking, which was associated with MM. They also presented analyses by days of use for individual pesticides. Approximately 9% of cases and controls reported use of glyphosate. ORs adjusted for smoking were 1.2 (95% CI 0.8–1.9) including all cases and controls and 1.1 (95% CI 0.7–1.9) excluding cases and controls who had proxy respondents. ORs excluding proxy respondents for one and two days/year of glyphosate use and for two or more days/year were 0.7 (95% CI 0.4–1.3) in the lower use category and 2.0 (95% CI 0.98–4.2) in the higher use category. However, these results for days of use per year were not adjusted for the potential confounding effects of other pesticides or farm exposures.

Assessment: The strengths of this study are the relatively large number of MM cases, the likelihood that almost all cases were confirmed histologically, and the explicit consideration of proxy respondents in the analysis. The limitations are likely residual confounding in the days of use per year analysis by the uncontrolled effects of other pesticides/exposures, selection bias (58% participation for cases and 48% participation for controls), and possible recall bias.

Sorahan (2015) conducted a re-analysis of data from the AHS to assess the basis for the disparate age-adjusted and

more fully adjusted glyphosate MM findings reported by De Roos et al. (2005). The author used Poisson regression to estimate RRs for MM in relation to glyphosate exposure categorized as ever versus never exposed and by levels of CEDs and IWEDs. Applicators who had missing covariate data were included in the analysis in a "not known" category so that the entire AHS cohort could be maintained. The RR for any glyphosate use adjusted for age and gender was 1.1 (95% CI 0.5–2.5); further adjusting for lifestyle factors and use of 10 other pesticides yielded a similar RR of 1.2 (95% CI 0.5–2.9). RRs for MM tended to increase with increasing CED and IWED reaching a peak RR of 1.9 (95% CI 0.7–5.3; p values for trend = 0.2) in the highest category of IWED in the fully adjusted model; however, none of the trend tests or category-specific RRs was statistically significant. This reanalysis showed that selection bias was associated with inflated MM risk estimates in the paper by De Roos et al. (2005). Those excluded from the analysis included five of eight MM cases in the glyphosate never use category. Sorahan's secondary analysis of this AHS data does not support the hypothesis that glyphosate use is a risk factor for MM and indicates that the practice of restricting analyses to subjects with complete data for all variables can produce appreciable bias.

Assessment: This reanalysis answers some of the questions about the impact of selection bias in the MM analysis by De Roos et al. (2005). Given that there were only 32 MM cases in the original publication, there are obvious limitations to analyses by estimated extent of exposure that can only be addressed with analyses of the AHS cohort using more recent follow-up data.

A special consideration: selection bias in the analysis

According to accepted case control theory (Rothman et al. 2008), the validity of case control studies depends on accurately estimating the exposure prevalence in the population that gave rise to the cases. Exposure prevalence cannot be estimated accurately by excluding from the analysis cases and controls with farm exposures other than glyphosate as was done in several studies. This practice distorts the glyphosate exposure prevalence for cases and controls and biases OR estimates. We illustrate this bias using data from such a glyphosate analysis by Brown et al. (1993).

Brown et al. (1993) analyzed a case control study that had 173 MM cases and 650 controls. Of these, 11 of 173 cases (6%) and 40 of 650 controls (6%) reported use of glyphosate. Hence, there was no difference in exposure prevalence for cases and controls. However, the authors calculated ORs using non-farmers as the referent population with the rationale that they were not exposed to any farm activities. This seemingly well-intentioned modification of the referent population violates a fundamental premise that underlies the validity of case control studies – that controls should be drawn from the population that gave rise to the cases, which, of course, includes individuals with exposure to farm activities. With these exclusions 100 of 173 cases (58%) and 338 of 650 controls (52%), the glyphosate exposure prevalence for cases was increased to 15% (11 of 73 cases) and the glyphosate exposure prevalence for controls was increased a lesser amount to 13% (40 of 312 controls). This created a bias away from the null as illustrated in Tables 5 and 6 in our OR analysis of the Brown et al. data with and without restriction of the referent group to those not exposed to any farm related activities (using Stata version 14).

Table 5. Results as presented by Brown et al. (1993) for glyphosate exposure.

	Case	Control	Total
Exposed	11	40	51
Unexposed	62	272	334
Total	73	312	385

$OR_{unadjusted}$ = 1.2, 95% CI 0.5, 2.6.

Table 6. Results for glyphosate exposure using all the cases and controls from Brown et al. (1993).

	Case	Control	Total
Exposed	11	40	51
Unexposed	162	610	772
Total	173	650	823

$OR_{unadjusted}$ = 1.0, 95% CI 0.5, 2.1.

Ironically, the reason for the clear bias away from the null is that those with exposure to farm related activities and who did not use glyphosate had higher MM risks than farmers who used glyphosate. In addition, by excluding those without exposure to glyphosate and exposure to other farm exposures, the authors would have precluded being able to control fully for confounding had they attempted multivariate analyses of pesticide exposures. Hardell et al. (2002), Eriksson et al. (2008) and Cocco et al. (2013) made similar exclusions, defining their referent population as those not exposed to pesticides (other than glyphosate). The limited data presented in those papers did not permit us to address statistically the direction and extent of the bias as we have for Brown et al. (1993).

In a similar vein, Sorahan's reanalysis of the MM data from the cohort analysis by De Roos et al. (2005) provides another example of selection bias in the analysis that produced an appreciable bias away from the null. In this case, Sorahan (2015) showed that excluding those with any missing covariate data increased the adjusted RR from 1.1 to 2.6, largely by excluding five of eight MM cases from the glyphosate unexposed population.

Weight of evidence evaluation

Descriptive summary

We systematically collected, summarized and critiqued 16 analytical epidemiological publications examining aspects of the possible relationship between reported use of glyphosate and two cancer types: NHL and MM. We excluded redundant publications (Cantor et al. 1992; Nordstrom et al. 1998; Hardell & Eriksson 1999; Pahwa et al. 2012) in favor of more recent published analyses of the same subjects. This resulted in a final evaluative dataset of seven studies of glyphosate exposure and NHL (see Table 2) and four studies of glyphosate exposure and MM (see Table 3), considering the Sorahan publication (2015) as an extension of De Roos et al. (2005).

The descriptive characteristics of each of these studies were examined for the likely presence or absence of validity concerns (see Table 4). It is clear from Table 4 that only one study in the glyphosate literature (highlighted in Table 4) – the AHS cohort study (De Roos et al. 2005) – was designed to minimize selection bias and recall bias, had only firsthand respondents reporting about exposures (viz. no proxy respondents), and conducted analyses that controlled comprehensively for confounding by personal characteristics and occupational exposures. In addition, the AHS cohort study was the only study that attempted to look at exposure–response relationships while controlling for confounding exposures. As such, it deserves the highest weight in our assessment of the literature. The other studies have so many validity concerns that they cannot be interpreted at face value. Indeed, there is evidence in many of these studies that virtually every exposure studied was associated with NHL or MM – a clear indication of widespread systematic bias and the unreliability of any of the reported exposure-disease associations.

We note one potential limitation to our systematic review. Although we were careful to systematically search the existing literature using search terms and secondary sources to identify relevant studies, it is possible that some relevant studies were not identified. Given the focus on glyphosate epidemiology by IARC and the authors of two recent meta-analyses, included among our secondary sources, we think this potential limitation is unlikely to be consequential.

Assessment of causality

The assessment of causality is a complex process that relies upon a family of well-recognized methods: the general scientific method (familiar to all scientists), study design and statistical methods, and research synthesis methods (e.g. the systematic narrative review, meta-analysis and pooled analysis, and the so-called criteria-based methods of causal inference). Of these, the criteria-based methods are often described and considered in causal assessments, with the most familiar having been proposed by Hill (1965) and utilized extensively in the 1964 Surgeon General's Committee on Smoking and Health and the many publications on the topic that dotted the scientific landscape in the late 1950s and early 1960s (Surgeon General 1964; Weed 2005). These "criteria" or "considerations" are substantive components of the stated methodologies of agencies such as the US EPA (2005) and IARC (2015).

At the center of these methods is the fundamental scientific aim of selecting the best explanation from the alternative explanations that exist for any body of scientific observations, however carefully they were obtained. In epidemiological terms, those alternative explanations typically are defined as cause, bias, confounding (a type of bias) and chance. Some studies are better at excluding alternative explanations than others; cohort studies, for example, are typically better at avoiding recall bias than interview based case-control studies, and recall bias affects not only the exposure of interest (here, glyphosate) but also potential confounding factors (e.g. exposure to other pesticides). Similarly, any and all epidemiologic study designs can – and should – control statistically for factors believed to be potential alternative explanations, i.e. known and putative confounders. For example, studying glyphosate and any lymphohematopoietic cancer without controlling for the potential confounding effects of other pesticides and herbicides, as was widely the case for almost all of the case control studies, does not permit one to exclude those confounders as an alternative explanation. And finally, if the results of an epidemiologic study (whether case-control or cohort) fail to achieve conventional levels of statistical significance – whether defined in terms of "*p* values" or "95% CIs" – then the alternative explanation of chance cannot be excluded. Notably, however, as Greenland (1990) pointed out, interpretation of *p* values and CIs at face value requires the assumption that a particular OR or RR has been estimated without bias (e.g. recall bias, selection bias, or confounding), elevating the importance of concerns about study validity in the interpretation of results.

In essence, all the causal frameworks in epidemiology focus on whether the observed associations are strong (viz. the size of the OR or RR is appreciably different than 1.0), whether the associations appear to have been estimated without bias, whether the OR or RR increases or decreases with increasing exposure (viz. exposure–response), whether the temporal relationship between exposure and effect is considered appropriate, and whether the results are statistically robust enough to rule out chance as an explanation (Hill 1965; Bhopal 2002; Aschengrau & Seage 2003a, 2003b; Sanderson et al. 2007).

Assessment of the NHL studies

With these considerations in mind, for NHL, it is justified scientifically to rely most on the results of the De Roos et al. (2005) cohort study as those best suited to reveal the existence (or not) of an association between exposure to glyphosate and NHL. This cohort study was the only study where information about pesticide use was collected independently of the participants' knowledge of cancer status, where there were no proxies providing information about pesticide use, where exposure–response was evaluated extensively, and where there was statistical adjustment for other pesticide exposures and personal factors in estimating RRs for glyphosate. As De Roos et al. (2005) concluded "… the available data provided evidence of no association between glyphosate exposure and NHL incidence." On the other hand, all the case control studies had the potential limitation of recall bias, many had clear indications of selection bias (either in terms of subject participation or in the analysis), most had very small numbers of glyphosate exposed cases and controls, none showed evidence of an exposure–response relationship, and most did not control for the potential confounding effects of personal factors or other occupational exposures in their glyphosate risk estimates. We consider the case control studies to be inadequate for the assessment of a relationship between glyphosate and NHL and consider the AHS cohort study as the one reliable evaluation of NHL risk from glyphosate. The two limitations of the AHS study are the relatively small number of NHL cases ($n = 92$) and that the length of follow-up after enrollment was less than

a decade. Those limitations speak to statistical robustness, not validity.

Assessment for MM

The glyphosate literature for MM is appreciably sparser than the literature for NHL. Again, the AHS cohort study (De Roos et al. 2005) is the best source of evidence when compared with the three available case control studies. The AHS data indicate that glyphosate users had about the same rate of MM as non-users adjusting for confounding factors (factoring in Sorahan's (2015) reanalysis of the fully adjusted MM results from De Roos et al. (2005) to correct the inadvertent selection bias discussed previously). Exposure–response analyses by De Roos et al. (2005) and Sorahan (2015) were relatively uninformative in light of the few MM cases split among exposure categories. More informative analyses await additional follow-up of the AHS cohort to increase the number of MM cases. The three MM case control studies are based on very small numbers, have concerns about recall bias and selection bias, and did not control for confounding by other exposures. Overall, then, we consider this literature inadequate to make an informed judgment about a potential relationship between glyphosate and MM.

Conclusions

The purpose of this literature review was to address two questions:

1. Does the current published epidemiologic evidence establish a causal relationship between glyphosate exposure and NHL?
2. Does the current published epidemiologic evidence establish a causal relationship between glyphosate exposure and MM?

Our review of the glyphosate epidemiologic literature and the application of commonly applied causal criteria do not indicate a relationship with glyphosate exposure and NHL. In addition, we consider the evidence for MM to be inadequate to judge a relationship with glyphosate. Our conclusion for NHL differs from that of the IARC workgroup seemingly because we considered the null NHL findings from the AHS to be more convincing than the case control studies, in aggregate, with their major limitations. We utilized a structured systematic review approach, we formally addressed pre-specified validity criteria for each study, and our weight of evidence assessment employed widely utilized criteria for causal inference.

Notes

1. A positive association has been observed between exposure to the agent and cancer for which a causal interpretation is considered by the Working Group to be credible, but chance, bias or confounding could not be ruled out with reasonable confidence.
2. Grey literature publications may include, but are not limited to the following types of materials: reports (pre-prints, preliminary progress and advanced reports, technical reports, statistical reports, memoranda, state-of-the art reports, market research reports, etc.), theses, dissertations, conference proceedings, technical specifications and standards, non-commercial translations, bibliographies, technical and commercial documentation, and official documents not published commercially (primarily government reports and documents) (Alberani et al. 1990).
3. Whether recall bias, exposure misclassification or selection bias was classified as likely or unlikely was based on a consensus after an in person discussion of each study by the authors.
4. According to accepted case control theory (see Rothman et al. 2008), the validity of case control studies depends on accurately estimating the exposure prevalence in the population that gave rise to the cases. Exposure prevalence cannot be estimated accurately by excluding from the analysis cases and controls with farm exposures other than glyphosate. This practice distorts the glyphosate exposure prevalence for cases and controls and biases OR estimates. We illustrate this in the section on selection bias in the analysis using data from such an analysis by Brown et al. (1993). In addition, excluding those with exposure to other pesticides hinders controlling for confounding by other farming exposures and pesticides in multivariate models.
5. Per footnote 2, defining the referent in this way distorts the glyphosate exposure prevalence for cases and controls, biases OR estimates, and precludes adequate control for confounding in multivariate models. See the section on selection bias in the analysis for additional details.
6. Per footnote 2, defining the referent in this way distorts the glyphosate exposure prevalence for cases and controls, biases OR estimates, and precludes adequate control for confounding in multivariate models. See the section on selection bias in the analysis for additional details.
7. Per footnote 2, defining the referent in this way distorts the glyphosate exposure prevalence for cases and controls, biases OR estimates, and precludes adequate control for confounding in multivariate models. See the section on selection bias in the analysis for additional details.

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Declaration of interest

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JA worked for Monsanto from 1989 through 2004 and is a consultant on a legal case unrelated to glyphosate that involves a former Monsanto industrial chemical plant. DG serves on a scientific advisory board to Dow Agro Sciences, which markets pesticides including glyphosate, and has consulted on behalf of Bayer Corp. on litigation matters concerning glyphosate and leukemia. GM has no additional declarations. TS has received consultancy fees and travel grants from Monsanto Europe SA/NV as a member of the European Glyphosate Toxicology Advisory Panel and participated in the IARC Monograph Meeting for volume 112, as an Observer for the Monsanto Company. In addition, TS has consulted for Monsanto on litigation matters involving glyphosate. DW has consulted on litigation matters concerning Monsanto that did not involve glyphosate.

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Supplemental material

Supplemental material for this article is available online here.

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REVIEW ARTICLE

OPEN ACCESS

Glyphosate rodent carcinogenicity bioassay expert panel review

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ABSTRACT

Glyphosate has been rigorously and extensively tested for carcinogenicity by administration to mice (five studies) and to rats (nine studies). Most authorities have concluded that the evidence does not indicate a cancer risk to humans. The International Agency for Research on Cancer (IARC), however, evaluated some of the available data and concluded that glyphosate probably is carcinogenic to humans. The expert panel convened by Intertek assessed the findings used by IARC, as well as the full body of evidence and found the following: (1) the renal neoplastic effects in males of one mouse study are not associated with glyphosate exposure, because they lack statistical significance, strength, consistency, specificity, lack a dose-response pattern, plausibility, and coherence; (2) the strength of association of liver hemangiosarcomas in a different mouse study is absent, lacking consistency, and a dose-response effect and having in high dose males only a significant incidence increase which is within the historical control range; (3) pancreatic islet-cell adenomas (non-significant incidence increase), in two studies of male SD rats did not progress to carcinomas and lacked a dose-response pattern (the highest incidence is in the low dose followed by the high dose); (4) in one of two studies, a non-significant positive trend in the incidence of hepatocellular adenomas in male rats did not lead to progression to carcinomas; (5) in one of two studies, the non-significant positive trend in the incidence of thyroid C-cell adenomas in female rats was not present and there was no progression of adenomas to carcinomas at the end of the study. Application of criteria for causality considerations to the above mentioned tumor types and given the overall weight-of-evidence (WoE), the expert panel concluded that glyphosate is not a carcinogen in laboratory animals.

Abbreviations: APVMA: Australian Pesticides and Veterinary Medicines Authority; EC: European Commission; EFSA: European Food Safety Authority; FAO: Food and Agriculture Organization; IARC: International Agency for Research on Cancer; ICH: International Conference on Harmonization; IPCS: International Program on Chemical Safety; JMPR: Joint FAO/WHO Meeting on Pesticide Residues; OECD: Organization for Economic Co-operation and Development; US EPA: United States Environmental Protection Agency; US FDA: United States Food and Drug Administration; WHO: World Health Organization

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Introduction

An expert panel was convened by Intertek, as described above (Williams et al. 2016) in response to the scientifically surprising conclusion of an International Agency for Research on Cancer (IARC 2015) panel's conclusion that data on glyphosate were sufficient to be classified by IARC as category 2a – "probably carcinogenic to humans". This conclusion contradicts a number of reviews and regulatory approvals that previously evaluated the carcinogenic and genotoxic potential of glyphosate (N-(phosphonomethyl)glycine) and its metabolite aminomethyl phosphonic acid. Glyphosate-based formulations (GBFs) were also in use prior to the

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development of IARC Monograph 112 (Health and Welfare Canada 1991; US EPA 1993a, 2013; WHO 1994; Williams et al. 2000; European Commission 2002; Kier & Kirkland 2013). The consensus among these reviews was that glyphosate was not considered to be an animal or human carcinogen and that the use of glyphosate and GBFs does not pose a genotoxic or carcinogenic hazard or risk. As a result, glyphosate-based herbicides have been approved for use in over 160 countries.

Background to the IARC evaluation

In this section, direct quotes from the IARC documentation are italicized so as to better define their stated objectives.

In examining what are called "agents", IARC refers to "specific chemicals, groups of related chemicals, complex mixtures, occupational or environmental exposures, cultural or behavioral practices, biological organisms and physical agents". A consistent pattern of consideration of this extraordinarily wide range of categories is clearly hard to achieve by a single mode of action (MoA).

Any of these categories might be considered in a *monograph,* which is stated to be the *first step in carcinogen risk assessment* – more precisely described as hazard identification. The monographs are intended to *identify cancer hazards even when* the perceived *risks are very low at current exposure levels, because new uses or unforeseen exposures could engender risks that are significantly higher*. In some IARC monographs, epidemiological studies used to identify a cancer hazard *can also be used to estimate a dose-response relationship*. The epidemiological review in the IARC document makes clear that this would not be appropriate regarding glyphosate.

IARC indicates that the outcome of these deliberations *represent only one part of the body of information on which public health decisions may be based*. It is nevertheless important that the data presented are the result of a set of deliberations, which acknowledge the characteristics of the scientific method in terms of the consideration of the available data.

Rodent carcinogenicity studies

Background

In considering any potential human carcinogen, information from many fields of science can be of value and none should be ignored, unless there are cogent and properly defined reasons for so doing. Studies that are poorly designed and thus inherently flawed may be excluded from consideration and developments in science subsequent to testing or new information may make it clear that the conclusions of earlier studies were not valid; this is how science progresses.

Animal testing over a significant portion of their lifespan is an integral part of the regulatory process and is clearly intended to provide information, which aids in the identification of potentially carcinogenic properties of a chemical. These properties are those that might result in an increased incidence of neoplasms in treated animals when compared with concurrent control groups. The studies may identify target organ(s) for carcinogenicity, characterize a tumor dose/response relationship, identify a no-observed-adverse-effect level (NOAEL) or point of departure for establishment of a benchmark dose, provide information allowing the extrapolation of carcinogenic effects to low-dose human exposure levels, and may also provide data to test hypotheses regarding a possible MoA (Williams et al. 2014).

Methods for evaluating the results of an extensive database of toxicology and carcinogenicity bioassays, as exist for glyphosate, have evolved from the application of WoE approaches (US EPA, 2005; Suter and Cormier, 2011) to approaches built on the systematic and rigorous methods of systematic evidence-based reviews (James et al. 2015). These approaches recommend that all reliable information be evaluated. Transparent descriptions of studies to be included and excluded are a key component of this approach. For example, if certain studies are determined to be invalid and thus not included, the reasons for these exclusions should be provided.

The majority of carcinogenicity studies are carried out in rodent species, most commonly with dosing *via* the oral route. In regulatory toxicology, the Organization for Economic Co-operation and Development (OECD) guidelines are commonly followed and these have been reviewed over a number of years, most recently in 2008 (OECD 2009). It therefore follows that in reviewing data on compounds that have been tested over many years, a careful examination of the precise nature of the studies reviewed must be made lest they fail to satisfy current standards of reliability. In any review, if any studies are to be ignored, the reasons for this should be provided.

The panel members were of the opinion that the IARC evaluation showed selectivity in the choice of data reviewed, with some omissions for which reasons were not clearly presented. These points will be considered below in more detail with regard to particular tumors, but an example of how an informative data set was not included in the IARC review is highlighted by the paper of Greim et al. (2015) who evaluated 14 carcinogenicity studies, nine chronic/carcinogenicity studies in the rat, including one peer-reviewed published study, and five carcinogenicity studies with glyphosate in mice. All were submitted to support glyphosate Annex I renewal in the European Union (European Commission, 2002) and were detailed in a supplement to the Greim et al. (2015) paper. The IARC Monograph reviewed only six rat and two mouse studies.

The dosing regimens in regulatory studies are determined on the basis of internationally agreed frameworks and in general, some evidence of an effect is sought. The attempt to demonstrate a potential toxic effect with a nontoxic compound, such as glyphosate has meant that the highest doses studied may utilize the compound at dosages of tens of thousands of parts per million in the diet, levels that are considered to be orders of magnitude greater than would be achieved from human exposure. Unusually, for glyphosate, there are also a number of studies in which lower doses are used.

Table 1 from Greim et al. (2015) provides a summary of the results of eight different rat studies conducted on glyphosate. As the studies used dietary exposure, the achieved dose levels in each study vary. Table 1 presents a tabulation of the

Table 1. Summary of select neoplasms in male rats (studies 1–8) listed in the legend*.

	Tumor incidence/number of animals examined by dose in mg/kg bw/day (ppm diet)											
Select neoplasm	Controls – 0 [range in %]	3† (30)	7.4§ (100)	10† (100)	10¶ (adjust)¶¶	31† (300)	73.9§ (1000)	86†† (1500)	89‡ (2000)	100¶ (adjust)¶¶	104# (3000)	121** (2000)
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	1/74	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

	Tumor Incidence/number of animals examined by dose mg/kg bw/day (ppm diet)													
Select neoplasm	150| (3000)	285†† (5000)	300¶ (adjust)¶¶	354# (10000)	361** (6000)	362† (8000)	740.6§ (10000)	780| (15000)	940‡ (20000)	1000¶ (adjust)	1077†† (15000)	1127# (30000)	1214** (20000)	1290| (25000)
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

The 25 doses result from the multiple doses per individual study.
*Taken from Greim et al. 2015.
†Study 1 (Monsanto) (CD) SD rats, rated unreliable for carcinogenicity evaluation.
‡Study 2 (Monsanto) (CD) SD rats, including interim sacrifice groups.
¶Study 3 (Cheminova) SD rats.
§Study 4 (Feinchemic Schwebda) Wistar rats.
|Study 5 (Excel) SD rats, rated unreliable for carcinogenicity evaluation.
#Study 6 (Arysta Life Sciences) Crj:CD SD rats, including interim sacrifice groups.
**Study 7 (Syngenta) Alpk:APfSD Wistar rats, including interim sacrifice groups.
††Study 8 (Nufarm) Wistar Han Crl:WI rats.
‡‡Recorded as parafollicular adenoma.
¶¶Dietary concentrations adjusted weekly to achieve target mg/kg bw/day dose.
NF: not found/not reported.

relevant tumor data for each of these eight studies in ascending order of achieved dose (lowest to highest). This allows a comparison of the incidence of specific neoplasms in each of the eight studies at all dose levels. As can be seen from Table 1, some of the benign tumors in male rats that appear to concern IARC in terms of the potential risk to humans, are widely represented in non-exposed animals as well as those exposed to doses well below those that might be expected in standard carcinogenicity studies conducted for regulatory purposes. The incidence of tumors shows no clear or consistent pattern, either across dose or individual study. Such a distribution of findings strongly indicates that these incidences represent spontaneous variations.

Neoplasm data can be analyzed using a survival-adjusted trend test that discriminates among fatal, incidental, and palpable neoplasms (Peto et al., 1980). If one or more tumor types in a valid bioassay show a significant positive trend in incidence rates, the significance level (p value) for rare ($\leq 1\%$ background incidence) neoplasms would be 0.025 and for common neoplasms 0.005 (US FDA 2001; Williams et al. 2014). For pairwise comparisons (control vs high dose), the significance of rare neoplasms would be 0.05 and of common 0.01 (US FDA 2001; Williams et al. 2014).

In the Monograph, IARC concluded that there is *sufficient evidence in experimental animals* for the carcinogenicity of glyphosate, reaching this opinion by the use of trend analysis in the absence of statistical significance in pairwise comparisons. Furthermore, the level of significance which differs between rare and common tumors was not taken into account.

Evaluation of IARC's conclusions

IARC concluded that glyphosate induced:

1. A significant positive trend in the incidence ($p = .037$) of renal tubule carcinomas and of adenomas and carcinomas ($p = .034$) in male CD-1 mice of one study only. This is a rare tumor type.
2. In a second feeding study in the same strain of mice, a significant positive trend in the incidence ($p < .001$) of hemangiosarcomas in male mice.
3. In two dietary studies in SD rats, a significant positive trend ($p < .05$) in the incidence of pancreatic islet cell adenomas occurred in male rats.
4. In the first dietary study in SD rats, a significant positive trend ($p = .016$) in the incidence of hepatocellular adenomas occurred in males.
5. In the first dietary study in SD rats, a significant positive trend ($p = .031$) in the incidence of thyroid C-cell adenomas occurred in females.

The expert panel evaluated each of these conclusions further below.

Kidney tubular-cell neoplasia in mice

The expert panel noted that the conclusions of the IARC monograph 112 (IARC 2015) with respect to kidney neoplasms in male CD-1 mice were based on only one of two oral mouse two-year carcinogenicity studies (Monsanto 1983; Cheminova 1993a) excluding two additional 18-month oral studies in CD-1 mice (Arysta Life Sciences 1997; Nufarm 2009), and one 18-month oral study in Swiss Albino mice (Feinchemie Schwebda 2001). All of the mouse studies were considered by expert groups to meet the guidelines for carcinogenicity bioassay in mice (US EPA 1990; ICH 1997). The two mouse studies evaluated by IARC, which were the first two studies reported, were also reviewed by Williams et al. (2000).

This section examines the renal neoplasms that occurred in the first two-year, oral chronic toxicity, and carcinogenicity study in CD-1 mice (Monsanto 1983), which was subsequently reevaluated by a pathology working group (PWG) (Dr. R M Sauer, Dr. MR Anver, Dr. JD Strandberg, Dr. JM Ward, and Dr. DG Goodman) and peer review experts including Dr. Marvin Kuschner M.D., Dean, School of Medicine, State University of New York at Stony Brook; Dr. Robert A. Squire, Robert A. Squire Associates Inc., Ruxton Maryland; Klaus L. Stemmer M.D., Kettering Laboratory, University of Cincinnati Medical Center, and; Robert E. Olson, M.D., Ph.D., Professor of Medicine and Pharmacological Sciences, State University of New York at Stony Brook (Sauer 1985; US EPA 1985a, 1985b, 1986, 1991a; McConnell 1986) and compares these findings to the other four chronic toxicity and carcinogenicity mouse studies with oral glyphosate (GLY) administration. These latter four studies did not produce renal neoplasms (Cheminova 1993a; Arysta Life Sciences 1997; Feinchemie Schwebda 2001; Nufarm 2009).

In the first two-year bioassay reported by Monsanto in 1983, male and female CD-1 mice were dosed with GLY at 0 (M0/F0, control group), 1000 [157/190, low-dose (LD) group], 5000 [814/955, mid-dose (MD) group] or 30,000 [4841/5874 mg/kg/d, high-dose (HD) group] ppm in the diet. In this and all the other carcinogenicity studies, HD animal survival was high. Some of the pertinent, but not significant, GLY-related effects were observed only in the high-dose group in males. They included: decrease in body weight gain, a centrilobular hepatocellular hypertrophy, and a urinary bladder hyperplasia. In addition, initially, neoplastic (benign) renal tubule adenomas were found microscopically in male mice only (0/49, 0/49, 1/50 (2%), 3/50 (6%) at the terminal necropsy. The initial diagnosis in one MD mouse (mouse #3023), and three HD mice (mouse #'s 4029, 4032, 4041) was that of renal cell adenoma (Monsanto 1983). This rare neoplasm is designated as renal cell adenoma or tubular cell adenoma (Greaves 2012). Macroscopically, the location and dimensions of these adenomas were as follows: In #3023, a mass was found on the right kidney (2.4×1.8 cm), in #4029, a very small area was suspected (no location and dimensions were given), in #4032, a suspicious area was found on the left kidney (0.5×0.4 cm), in #4041, a suspicious area was found on the left kidney (0.6 cm in diameter). Subsequently, reevaluation was made by a PWG that resulted in a report by Sauer (1985) and McConnel (1986). This was also reflected in four US EPA submissions (US EPA 1985a, 1985b, 1986, 1991a). The final evaluation of the

Table 2. Final evaluation of pertinent renal histopathology findings from Monsanto Study (1983).

Diagnosis	Mouse and group identification	Group incidence
Tubular-cell adenoma	1028	Control 1/49
Tubular-cell carcinoma	**3023***,	Mid dose 1/50
	4029, 4032, 4041	High dose 3/50
Tubular-cell hyperplasia	1018,	Control 1/49
	3031, 3039,	Mid dose 2/50
	4008, 4049	High dose 2/50
Intercurrent† papillary hyperplasia	1008, 1041,	Control 2/49
	3008, 3050	Mid dose 2/50

Bold numbers indicate the original histopathological diagnosis of tubular-cell adenoma in four male mice; TCA/TCC, this combination was utilized in the IARC 2015 evaluation, only the trend analysis was $p = .034$, a value level, which is not significant for rare tumors (US FDA 2001); *, this neoplasm was the largest of all neoplasms; †, intercurrent occurring indicates while another process/renal toxic change was in progress.

kidney pathology produced the following incidences of pertinent renal findings detailed in Table 2.

The overall final incidence of renal neoplasms in male mice was as follows: 1/49, 0/49, 1/50, and 3/50, with the largest neoplasm being in the MD (#3023) group. The important non-neoplastic renal findings of hyperplasia were as follows: 3/49, 0/49, 4/50, and 2/50, indicating absence of a dose-response, with the highest incidence in the MD group, followed by the control group, and the HD group. The LD group had no renal findings.

Based on the pattern of pre-neoplasia and neoplasia described above, the PWG recommendation was that the renal neoplasms were not compound related, since they were not preceded by dose-related proliferative changes (hyperplasia). Thus, there was no dose-response for pre-neoplasia. In addition, no multiple renal neoplasms and no nephrotoxic lesions were found in any of the mice; many mice had proliferative/cystic lesions in the parietal layer of the Bowmans' capsule and proximal convoluted tubules. These changes, however, were more severe in controls. In addition, the females from the HD group of the study had no renal neoplasms and only proximal tubule epithelial basophilia and hypertrophy. No discrepancies were noted in any of the histopathology reporting among the various expert panel groups (Sauer 1985; US EPA 1985a, 1985b, 1986; McConnel 1986).

In addition to the PWG recommendations (above), a review of the renal lesions, which occurred only in 14 out of 198 male mice at the termination of the first (Monsanto 1983) study, showed clearly that none of the occurrences of hyperplasia (tubular-cell hyperplasia or intercurrent papillary hyperplasia were present in mice that had tubular-cell adenoma or tubular-cell carcinoma (Table 2). The absence of hyperplasia indicates that all renal proliferative and neoplastic lesions occurred *de novo* in male mice in all experimental groups (including controls), i.e. they were spontaneous or background lesions, and were not compound related. Moreover, the female mice, which had received 1.2-times, 1.1-times, or 1.2-times more GLY, within the LD, MD, or HD groups, respectively, had no renal neoplastic lesions.

Thus, the Monsanto (1983) report concluded that for male and female mice, the lower NOAEL was 157 mg/kg/d, and the lowest observed adverse effect level was 814 mg/kg/d.

Three additional oral carcinogenicity studies were conducted in CD-1 mice and one in Swiss Albino mice (Cheminova 1993a; Arysta Life Sciences 1997; Feinchemie Schwebda 2001; Nufarm 2009).

The Cheminova (1993a) report, was a two-year mouse study. In this study, no renal neoplasms were evident up to 1000 mg/kg/d (HD) of GLY in CD-1 mice of both sexes.

In an 18-month diet study in CD-1 mice, histopathological evaluations of groups dosed up to 4200 mg/kg/d of GLY (HD), did not show any evidence of renal neoplasms in male or female mice (Arysta Life Sciences 1997).

In an 18-month diet study in Swiss Albino mice, up to 1460 mg/kg/d (HD) of GLY produced no statistically significant neoplastic lesions (Feinchemie Schwebda 2001) and finally, in a 18-month diet study in CD-1 mice at dosages up to 946 mg/kg/d (HD) of GLY was shown not to be carcinogenic to the kidney (Nufarm 2009).

In the last four mouse carcinogenicity studies, multiple-section sampling of kidneys for histopathology was utilized according to Eustis et al. (1994).

Thus, for the five glyphosate mouse carcinogenicity studies, only the first conducted study showed any neoplastic renal lesions and these occurred only in male mice of the MD at 814 mg/kg/d, and HD groups at 4841 mg/kg/d. All of these general and renal neoplastic findings indicating a lack of a glyphosate renal carcinogenic response were reported in key regulatory submission updates (US EPA 1985a, 1985b, 1986, 1991a, 1993a, 1993b, 2012, 2013; JMPR 1987, 2006, 2014, 2016; IPCS 1996, 2005; European Commission 2002; EFSA 2009, 2015), and one review publication (Greim et al. 2015).

In conclusion, 14 GLY carcinogenicity studies (nine rat and five mouse) were evaluated for their reliability, and selected neoplasms were identified for further evaluation across all databases (Greim et al. 2015). The mouse renal neoplasms occurred only in males of the first study. In the other four, the HD of 1000 mg/kg/d (Cheminova 1993a), 4200 mg/kg/d (Arysta Life Sciences 1997), 946 mg/kg/d (Nufarm 2009), and 1460 mg/kg/d (Feinchemie Schwebda 2001) produced no renal neoplasms in either male or female mice.

The assessment of this study (Monsanto 1983) based on the PWG of the US EPA (1986) evaluation and which was reported by IARC (2015), concluded that the incidence of renal tubule adenoma: 1/49 (2%), 0/49, 0/50, 1/50 (2%), was not statistically significant, whereas, the incidence of renal tubule carcinoma: 0/49, 0/49, 1/50 (2%), 2/50 (4%), was significant at $p = .037$ (in the Cochran–Armitage trend test). When the adenomas and carcinomas were combined: 1/49 (2%), 0/49, 1/50 (2%), 3/50 (6%), then the value was $p = .034$ (in the Cochran–Armitage trend test). While both these p values ($p = .037$ and $p = .034$) were reported to be significant in this one study, it is important that these p values are not considered significant for rare neoplasms, for which authorities require a level of significance for trend at $p < .025$ (US FDA 2001).

Furthermore, the Panel applied to the kidney neoplasms noted within the Monsanto (1983) study a set of logical considerations for causation similar to those proposed for evaluation of epidemiologic data (Hill, 1965; Woodside & Davis, 2013) to assess whether an association between exposure

and effect (two variables) might be deemed strong, consistent, specific, temporal, plausible, coherent, and to demonstrate a dose-response pattern. Several conclusions following this evaluation were made:

1. The association is not strong, since the higher incidences of rare renal neoplasms in dosed groups are not considered to be statistically different from control group.
2. The association is not consistent, since four out of five mouse studies did not reproduce similar renal neoplasms at comparable doses.
3. The association is not specific, since females of this pivotal study, which have been exposed to higher levels of GLY did not develop renal neoplasms. Also, there were no renal findings (hyperplasia or neoplasia) in the LD group, whereas the control group had four incidences of hyperplasia or adenoma (Table 2).
4. The time required between exposure and effect, i.e. a reduced latency time was not present; all tumors were observed only at termination. Also, no mouse with neoplasia had also hyperplasia, and the largest tubular-cell carcinoma (#3023) was in the MD group.
5. The biological gradient of association or the dose-response curve was absent, since the females and the males in LD group had no neoplasms, whereas the controls had one.
6. A plausible explanation for the association was absent, since a MoA for induction of these renal neoplasms was not established.
7. Coherence of the association was also absent, female mice and male and female rats did not display kidney effects. Also, in the other four mouse carcinogenicity studies the mice did not develop similar neoplastic renal lesions.
8. The association does not demonstrate a dose-response pattern (see #5, 6), since the "in-study" females had neither neoplasms nor any of the other renal lesions, although they were exposed to higher levels of GLY.

Hemangiosarcomas in mice

This is a common neoplasm in this strain of mice with historical control values for both males and females ranging from 2 to 12%. This tumor was observed only in the liver.

The IARC conclusion was that "there was a significant ($p < .001$) positive trend in the incidence of hemangiosarcoma in high dose male CD-1 mice" (Control 0%, 0%, 0%, 8%) based on their interpretation of the Joint Meeting of the FAO panel of experts on Pesticide Residues in Food and the Environment (JMPR) 2006 study. Yet in females, the highest incidence (4%) was in the low-dose group followed by the high dose (2%) (Table 3).

In the CD-1 mouse study reported by Cheminova (1993a), the animals were fed diets providing intakes of glyphosate at dose levels of 100, 300, or 1000 mg/kg bw/d for 104 weeks. There were no treatment related effects on survival or body weight, nor were there any notable intergroup differences in the incidences of externally palpable masses. There were no statistically significant increases in the incidence of any tumors when compared with the control groups and no dose response was evident.

Based on their own statistical analysis, IARC concluded that there was an increase in the incidence of hemangiosarcoma in males [$p < .001$, Cochran–Armitage trend test].

IARC did not comment on the absence of hemangiosarcomas in Nufarm (2009), an 18-month diet study in CD-1 mice providing intakes up to 946 mg/kg bw/d of glyphosate similar to the previous study high dose. IARC also failed to note the historical control data, which have a range of 2–12% for both sexes (Charles River Labs 2000). Therefore, the statistically significant tumors were within the control data range (Table 3).

If the likelihood of the occurrence of hemangiosarcoma is considered in terms of the criteria for causality, it is clear that there is no strength in the association. For example, pairwise comparisons are not significant, there is no consistency (other mouse studies show no tumors of this type at all), a dose/response effect was not seen (some HD groups have a lower incidence than lower dose groups). In addition, the dose (about 170 mg/kg bw/d) associated with the highest incidence in males, did not produce any renal neoplasia in this study. Moreover, the female mice which have received higher doses of GLY had no significant incidence of hemangiosarcomas. Thus, despite the significantly positive trend in high dose males only, the incidence of this neoplasm was not compound related.

Pancreatic tumors in rats

Pancreatic islet cell tumors are common in this strain of rat (Williams et al. 2014). In two of the nine carcinogenicity studies in rats evaluated by IARC, tumors of islet cells of the pancreas were diagnosed in both males and females. Both studies were made available to IARC by the US EPA (1991a, 1991b, 1991c).

In the first study, SD rats received 0, 30 (3), 100 (10), and 300 (31 mg/kg bw/d) ppm *ad libitum* in diet for 26 months. No pancreatic islet carcinomas were observed. The incidence of adenoma was found to have a positive trend ($p < .05$) in the study. However, the level of significance for common tumors should be $p < .005$. The following islet cell adenoma

Table 3. Incidences of hemangiosarcoma in CD-1 mouse study (Cheminova 1993b).

	Tumor incidence/number of animals examined (mg/kg bw/d)*							
	Males				Females			
	0	100	300	1000	0	100	300	1000
Hemangiosarcomas	0/50	0/50	0/50	4/50 (8%)	0/50	2/50 (4%)	0/50	1/50 (2%)

*Taken from Greim et al. (2015) supplemental data, doses were administered in the diet, with dietary concentrations adjusted regularly to achieve target mg/kg bw/day dose.

Table 4. Liver tumor incidences/number of Sprague–Dawley rats/group (Stout and Ruecker 1990).

	Males				Females			
mg/kg bw/d (ppm)	0 (0)	89 (2000)	362 (8000)	940 (20,000)	0 (0)	113 (2000)	457 (8000)	1183 (20,000)
Interim sacrifice (12th month)								
Hepatocellular adenoma	0/10	0/10	0/10	0/10	0/10	0/10	1/10	0/10
Hepatocellular carcinoma	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
Unscheduled deaths								
Hepatocellular adenoma	2/36	1/31	0/33	4/33	0/28	1/28	2/33	1/32
Hepatocellular carcinoma	2/36	1/31	1/33	2/33	0/28	0/28	0/33	1/32
Scheduled sacrifices								
Hepatocellular adenoma	1/14	1/19	3/17	4/17	6/22	1/22	3/17	0/18
Hepatocellular carcinoma	1/14	1/19	0/17	0/17	1/22	0/22	1/17	1/18
All deaths								
Hepatocellular adenoma	3/60	2/60	3/60	8/60	6/60	2/60	6/60	1/60
Hepatocellular carcinoma	3/60	2/60	1/60	2/60	1/60	0/60	1/60	2/60

incidences were observed for controls, low, mid and high doses respectively in males: 0/50, 5/49 (10%), 2/50 (4%), 2/50 (4%). This incidence data shows no dose-response patterns and preneoplastic effects are absent. In addition, in the first study in males, the adenomas also did not progress to carcinomas. Thus, the pancreatic islet cell adenomas were not compound-related. In females, the corresponding values were: 2/50 (4%), 1/50 (2%), 1/50 (2%), and 0/50.

In the second study, male and female Sprague–Dawley (SD) rats were fed 0, 2000 (89/113), 8000 (362/457), or 20,000 (940/1183 mg/kg bw/d) ppm glyphosate (96.5% pure) *ad libitum* in diet for 24 months. The following islet cell tumor incidences were observed in males: adenomas – 1/58 (2%), 8/57 (14%), 5/60 (8%), 7/59 (12%); carcinomas – 1/58 (25%), 0/57, 0/60, 0/59. In females, the corresponding incidences were: adenomas – 5/60 (8%), 1/60 (2%), 4/60 (7%), 0/59; carcinomas – 0/60, 0/60, 0/60, 0/59. The historical control rates for pancreatic islet cell tumors at the testing laboratory were in the range 1.8–8.5%. The panel disagrees with the conclusion of IARC that there is a significant positive trend ($p < .05$) in the incidence of pancreatic adenomas in males, since the level of significance for trend should be $p < .005$ (US FDA 2001; Williams et al. 2014). Moreover, there was no progression of adenomas to carcinomas.

Four additional studies in rats, described by Greim et al. (2015), but not evaluated by IARC, similarly did not show pancreatic islet cell tumors. Based on this information, the panel concluded that there is no evidence that glyphosate induces islet cell neoplasia in the pancreas.

Liver tumors in rats

Hepatocellular neoplasms are common for this strain of rat (about 5% in males and 3% in female controls) (Williams et al. 2014). The IARC evaluation indicated that there was "…a significant positive trend ($p = .016$) in the incidences of hepatocellular adenoma in males…" (IARC 2015). This opinion was based on its interpretation of the Stout and Ruecker (1990) study as presented by the US EPA's Peer Review of Glyphosate (US EPA 1991b, 1991c).

In the Stout and Ruecker (1990) carcinogenic bioassay, SD rats were exposed through the diet to 0, 2000, 8000, and 20,000 ppm of 96.5% pure glyphosate for 24 months. These dietary concentrations corresponded to 0, 89, 362, and 940 mg/kg bw/d for males and 0, 113, 457, and 1183 mg/kg bw/d for females, the highest tested dose (HTD) being close to the limit dose for long-term studies with rats (OECD 2009). No glyphosate-related clinical signs or influence on survival were observed. At term, there was no influence on body weights or body weight gain by males; in the females there was a 6.4% decreased body weight gain. The original data on tumor incidence in this study are available in Greim et al. (2015). The all-deaths incidences of hepatocellular adenomas or carcinomas in the glyphosate-exposed groups were not significantly different from the controls (Table 4). At the 12th month (interim sacrifice), no adenomas or carcinomas were observed in the male groups, but a single adenoma case was noted in a female at 457 mg/kg/d. The rates of hepatocellular adenomas in females and of hepatocellular carcinomas in each sex followed no dose-response pattern at any time. In males, the first liver adenoma and carcinoma were observed at week 88 and 85, respectively, in animals exposed to the HTD of 940 mg/kg/d. A non-significant numerically greater ($p = .101$, Fisher Exact) incidence of hepatocellular adenomas occurred in male rats exposed to the highest dose, since it is a common tumor type, the level of significance required is $p < .01$. There was no progression from adenoma to carcinoma. The authors did not highlight the occurrence of hepatocellular tumors in their final report and concluded that "an oncogenic effect was not observed".

The Stout and Ruecker (1990) study has been reviewed twice by the US EPA (1991b, 1991c). The US EPA memoranda indicate that the incidences of hepatocellular adenomas in males were within the range (1.4–18.3%) of historical controls from the Monsanto Environmental Health Laboratory (EHL), where the study was conducted. Additional statistical analyses developed by US EPA on liver tumor rates of male rats surviving after the 55th week indicated that the incidence of adenomas in the HTD males did not differ significantly from the control by the Fisher's Exact Test pair-wise comparison, but detected a significant trend ($p = .016$) by the Cochran–Armitage trend test (see also above) (Table 5). Since liver adenoma is a common tumor type, the significance level for trend should be 0.005 (US FDA 2001; Williams et al. 2014). It should be noted that the incidences of hepatocellular adenomas in animals exposed to the two intermediate doses were of the same magnitude as the controls, i.e. there was no linear ascending trend of incidence across doses, but a "hockey-stick"-type slope. The biological importance of the

Table 5. Sprague–Dawley male rats: hepatocellular tumor rates† and Cochran–Armitage trend and Fisher's exact tests results (*p* values).

	Dose mg/kg bw/d (ppm)			
Tumors	0 (0)	89 (2000)	362 (8000)	940 (20,000)
Carcinomas	3/34	2/45	1/49	2/48‡
(%)	(7)	(4)	(2)	(4)
p	.324	.489	.269	.458
Adenomas	2/44	2/45	3/49	7/48¶
(%)	(5)	(4)	(6)	(15)
p	**.016***	.683	.551	.101
Adenoma + Carcinoma	5/44	4/45	4/49	9/48
(%)	(11)	(9)	(8)	(19)
p	.073	.486	.431	.245
Hyperplasia only	0/44	0/45	1/49§	0/48
(%)	(0)	(0)	(2)	(0)
p	.462	1.000	.527	1.000

Adapted from Table 3 (US EPA 1991a) or Table 7 (US EPA 1991b).
*($p < .05$) Significance of trend indicated at control (0 ppm). Significance of pair-wise comparison with control denoted at dose level, if occurred.
†Number of tumor-bearing animals/number of animals examined, excluding those that died or were sacrificed before week 55.
‡First carcinoma observed at week 85 at 20,000 ppm;
¶First adenoma observed at week 88 at 20,000 ppm;
§First hyperplasia observed at week 89 at 8000 ppm.

observed data should be taken into account (OECD 2012) and in this case the result of the trend test should not override the absence of significance found by the pair-wise test.

The final interpretation of the US EPA Review committee was appropriate: "Despite the slight dose-related increase in hepatocellular adenomas in males, this increase was not significant in the pair-wise comparison with controls and was within the historical control range. Furthermore, there was no progression from adenoma to carcinoma and incidences of hyperplasia were not compound-related. Therefore, the slight increased occurrence of hepatocellular adenomas in males is not considered compound-related" (US EPA 1991b). As noted previously, the US EPA ultimately concluded that glyphosate should be classified as a Group E (evidence of non-carcinogenicity for humans) chemical (US EPA 1991b, 1991c).

There are other aspects of the Stout and Ruecker (1990) data that support the conclusion that glyphosate did not exert an oncogenic effect on the liver of SD rats. For example, chemical-induced rat hepatocellular carcinogenesis is a multiple stage process characterized by progressive functional, morphological and molecular changes that indicate or precede the full establishment of neoplasia, such as enzyme induction, hepatocyte hypertrophy, degeneration and necrosis, hepatocyte proliferation, hyperplasia, and preneoplasia, i.e. altered hepatocellullar foci, and malignant tumors (Williams 1980; Bannasch et al. 2003; Maronpot et al. 2010). Identification and analyses of these liver changes – that span from adaptative to irreversible adverse effects – can support characterization of key events along the carcinogenesis process and inform the MoA of the tested chemical (Williams & Iatropoulos 2002; Holsapple et al. 2006; Carmichael et al. 2011). None of these alterations were significantly found in this study.

It is clear that there was a non-significant numerically greater incidence of liver adenomas in a long-term bioassay with male rats exposed to glyphosate, at a dose that was close to the limit dose. There was no progression to malignancy and no compound-associated pre-neoplastic lesions were induced.

Table 6. Tumor Incidence/number of animals examined (mg/kg bw/d) (Stout and Ruecker 1990 all deaths reported).

	Males				Females			
	0	89	362	940	0	113	457	1183
Thyroid C cell adenoma	2/60	4/58	8/58	7/60	2/60	2/60	6/60	6/60
Thyroid C cell carcinoma	0/60	2/58	0/58	1/58	0/60	0/60	1/60	0/60

In the last 30 years, the systemic carcinogenic potential of glyphosate has been assessed in at least eight studies in Sprague–Dawley or Wistar rats (Greim et al. 2015); a ninth could not be evaluated because of a high mortality and the low doses used (Chruscielska et al. 2000). Considered jointly, these animals were exposed through the diet to 24 different doses distributed across a wide range of 3.0–1290.0 mg/kg bw/d. In exposed males, the incidences of hepatocellular adenomas across the doses showed no dose-response relationship and varied within the same range as the controls. Similar rates were also seen for hepatocellular carcinomas. These observations confirm the absence of carcinogenic potential of glyphosate on the rat liver.

Thyroid tumors in rats

C-cell tumors of the thyroid are a common tumor in this strain of rat (Williams et al. 2014).

The incidence of thyroid C-cell adenoma in females was reported in the Monograph (IARC 2015) to have a significant positive trend ($p = .031$). IARC based their opinion, again, on its interpretation of the Stout and Ruecker (1990) study and the US EPA's Second Peer Review of Glyphosate (US EPA 1991a).

In the Stout and Ruecker (1990) study, no statistically significant difference was reported in the incidence of thyroid C-cell neoplasms, as shown in Table 6. Additionally, the US EPA (1991a) concluded that "the C-cell adenomas in males and females are not considered compound-related." Although the C-cell adenomas were slightly numerically greater in male and female mid- and high-dose groups, there was no dose related progression to carcinoma and no significant dose-related increase in severity of grade or incidence of hyperplasia in either sex. However, IARC concluded that "there was also a statistically significant positive trend in the incidence of thyroid C-cell adenoma in females ($p = .031$)." But, because this is a common tumor type, the trend significance value should be $p < .005$ (US FDA 2001; Williams et al. 2014). Thus, the incidence of this tumor is not statistically significant.

In the Arysta Life Sciences (1997) study, no increase in C-cell adenomas up to 1247 mg/kg/d was reported. The Chruscielska et al. (2000) study in Wistar rats is not informative and this work fails to meet appropriate standards for inclusion.

Thus, in one of the two studies, the significant trend in the incidence of thyroid C-cell adenomas in female rats did not materialize, although the adenomas were only slightly increased in mid and high doses, but there was no progression to malignancy. Thus, only one out of nine life-time

studies in rats showed a slight not significant increase in C-cell adenomas, which however did not progress to carcinomas.

Evaluations by regulatory agencies, scientific bodies and third party experts

A number of scientific groups, regulatory agencies and individuals have evaluated and commented on these data with the latter grouping from third party experts appearing in peer reviewed documents. The expert panel agrees with the opinions expressed below that glyphosate was not carcinogenic to rodents.

Regulatory agencies

- EFSA 2015: "No evidence of carcinogenicity was confirmed by the large majority of the experts (with the exception of one minority view) in either rats or mice due to a lack of statistical significance in pair-wise comparison tests, lack of consistency in multiple animal studies and slightly increased incidences only at dose levels at or above the limit dose/maximum tolerated dose, lack of preneoplastic lesions and/or being within historical control range. The statistical significance found in trend analysis (but not in pair-wise comparison) *per se* was balanced against the former considerations." (EFSA 2015)
- APVMA (2013) – "The weight and strength of evidence shows that glyphosate is not genotoxic, carcinogenic, or neurotoxic."
- US EPA (2013) – "No evidence of carcinogenicity was found in mice or rats."
- US EPA (2012) – "No evidence of carcinogenicity was found in mice or rats."
- European Commission (2002) – "No evidence of carcinogenicity."
- US EPA (1993a, 1993b) – "The Agency has classified glyphosate as a Group E carcinogen (signifies evidence of non-carcinogenicity in humans)."
- Health and Welfare Canada (1991) – "Health and Welfare Canada has reviewed the glyphosate toxicology data base, which is considered to be complete. The acute toxicity of glyphosate is very low. The submitted studies contain no evidence that glyphosate causes mutations, birth defects or cancer."

Scientific bodies

- JMPR (2016) – "Glyphosate is not carcinogenic in rats, but could not exclude the possibility that it is carcinogenic in mice at very high doses."
- JMPR (2006) – "In view of the absence of a carcinogenic potential in animals and the lack of genotoxicity in standard tests, the meeting concluded that glyphosate is unlikely to pose a carcinogenic risk to humans."
- WHO (1994) – "The available studies do not indicate that technical glyphosate is mutagenic, carcinogenic or teratogenic."
- JMPR (1987) – "The chronic toxicity of glyphosate is low … There is no evidence of carcinogenicity."

Independent experts

- Williams et al. (2000) – "It was concluded that, under present and expected conditions of use, Roundup herbicide does not pose a health risk to humans."
- Greim et al. (2015) – "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."

Conclusions

After review of all available glyphosate carcinogenicity data, the panel concluded:

i. The rare renal tubule tumors in one male (CD-1) mouse study were not associated with glyphosate exposure, because they lacked statistical significance, strength, consistency, specificity, dose-response patterns, plausibility, and coherence.
ii. In a different mouse (CD-1) study, there was a lack of association of exposure to glyphosate and a statistically significant positive trend for the incidence of liver hemangiosarcoma (a common tumor) because the findings were inconsistent, there was no dose-response effect, and the incidences were within the historical control range.
iii. The strength of association of pancreatic islet-cell adenomas (a common tumor) to glyphosate exposure in two studies of male SD rats was absent. There was a lack of a dose-response pattern (the highest incidence is in the low dose followed by the high dose), plausibility and absence of pre-neoplastic effects and progression to islet-cell carcinomas.
iv. In one of two studies, a significant positive trend in the incidence of hepatocellular adenomas (a common tumor) in male SD rats did not occur, and no progression to carcinomas was evident and no glyphosate-associated pre-neoplastic lesions were present.
v. In one of two studies, the significant positive trend in the incidence of thyroid C-cell adenomas in female SD rats was not evident. The adenomas were only slightly increased in mid and high doses, within the historical ranges. Also, there was no progression to carcinomas.

Application of criteria for causality considerations to the above mentioned tumor types and given the overall WoE, the expert panel concluded that glyphosate is not a carcinogen in laboratory animals.

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Declaration of interest

The employment affiliation of the authors is as shown on the cover page. However, it should be recognized that each individual participated in the review process and preparation of this paper as an independent professional and not as a representative of their employer.

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Intertek (previously Cantox) is a consultancy firm that provides scientific and regulatory advice, as well as safety and efficacy evaluations for the chemical, food, and pharmaceutical industries. While Intertek has not previously worked on glyphosate related matters for the Monsanto Company, previous employees (Ian Munro, Barry Lynch) of Cantox, have worked in this capacity. These employees of Cantox, and Gary Williams, prepared a safety and risk assessment, including the carcinogenicity, of Roundup herbicide (glyphosate), which was published in 2000 (Williams GM, Kroes R, Munro IC (2000). Safety evaluation and risk assessment of the herbicide roundup and its active ingredient, glyphosate, for humans. Regul Toxicol Pharmacol 31(2):117–165).

Gary Williams, Sir Colin Berry, João Lauro Viana de Camargo, and Helmut Greim have previously served as independent consultants for the Monsanto Company, some on the European Glyphosate Task Force. Gary Williams has consulted for Monsanto on litigation matters involving glyphosate. Michele Burns has not previously been employed by the Monsanto Company or previously been involved in any activity involving glyphosate and as such declare no potential conflicts of interest. Furthermore, other than Gary Williams, none of the aforementioned authors have been involved in any litigation procedures involving glyphosate.

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Genotoxicity Expert Panel review: weight of evidence evaluation of the genotoxicity of glyphosate, glyphosate-based formulations, and aminomethylphosphonic acid

David Brusick, Marilyn Aardema, Larry Kier, David Kirkland & Gary Williams

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REVIEW ARTICLE

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Genotoxicity Expert Panel review: weight of evidence evaluation of the genotoxicity of glyphosate, glyphosate-based formulations, and aminomethylphosphonic acid

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ABSTRACT

In 2015, the International Agency for Research on Cancer (IARC) published a monograph concluding there was strong evidence for genotoxicity of glyphosate and glyphosate formulations and moderate evidence for genotoxicity of the metabolite aminomethylphosphonic acid (AMPA). These conclusions contradicted earlier extensive reviews supporting the lack of genotoxicity of glyphosate and glyphosate formulations. The IARC Monograph concluded there was strong evidence of induction of oxidative stress by glyphosate, glyphosate formulations, and AMPA. The Expert Panel reviewed the genotoxicity and oxidative stress data considered in the IARC Monograph, together with other available data not considered by IARC. The Expert Panel defined and used a weight of evidence (WoE) approach that included ranking of studies and endpoints by the strength of their linkage to events associated with carcinogenic mechanisms. Importantly, the Expert Panel concluded that there was sufficient information available from a very large number of regulatory genotoxicity studies that should have been considered by IARC. The WoE approach, the inclusion of all relevant regulatory studies, and some differences in interpretation of individual studies led to significantly different conclusions by the Expert Panel compared with the IARC Monograph. The Expert Panel concluded that glyphosate, glyphosate formulations, and AMPA do not pose a genotoxic hazard and the data do not support the IARC Monograph genotoxicity evaluation. With respect to carcinogenicity classification and mechanism, the Expert Panel concluded that evidence relating to an oxidative stress mechanism of carcinogenicity was largely unconvincing and that the data profiles were not consistent with the characteristics of genotoxic carcinogens.

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Executive summary

Overall, extensive reviews of the genotoxicity of glyphosate, aminomethylphosphonic acid (AMPA) and glyphosate based formulations (GBFs) that were available prior to the development of the International Agency for Research on Cancer (IARC) Glyphosate Monograph all support a conclusion that glyphosate (and related materials) is inherently not genotoxic. Further, evidence indicative of an oxidative stress mechanism of carcinogenicity is largely unconvincing. The Expert Panel concluded that there is no new, valid evidence presented in the IARC Monograph that would provide a basis for altering these conclusions.

The differences between the conclusions of the IARC review and the Expert Panel review were in large part due to IARC exclusion of numerous available studies and in some cases differences in interpretation of study results reported in the IARC Monograph. Another significant source of difference was the Expert Panel's weighting of different studies and endpoints by the strength of their linkage to mutagenic events associated with carcinogenic mechanisms. The Expert Panel concluded that without critically evaluating all available data, it is not possible to make an accurate weight of evidence (WoE) assessment.

The IARC review process does not allow for use of data from reports that are not published or accepted for publication in the open scientific literature or data from government reports that are not publicly available. However, detailed primary data were extracted and published in reviews such as Kier and Kirkland (2013), although the study reports themselves are unpublished. The Expert Panel concluded that these data along with regulatory studies of GBFs and AMPA summarized in Williams et al. (2000) should have been considered by IARC, and should be considered by all stakeholders going forward in evaluating the genetic toxicology of glyphosate and GBFs. A critical review of the complete dataset by the Expert Panel supports a conclusion that glyphosate (including GBFs and AMPA) does not pose a genotoxic hazard and therefore, should not be considered support for the classification of glyphosate as a genotoxic carcinogen.

Introduction

In 2015, IARC published the Glyphosate Monograph of Volume 112 (IARC 2015) which concluded that there was strong evidence supporting that "glyphosate can operate through two key characteristics of known human carcinogens" including genotoxicity and induction of oxidative stress. This was viewed as providing strong support for IARC classifying glyphosate as probably carcinogenic to humans, Group 2A. A number of published and regulatory approval reviews of the carcinogenic and genotoxic potential of glyphosate, AMPA and GBFs were available prior to the development of the IARC Monograph (Health and Welfare Canada 1991; US EPA 1993; WHO 1994; Williams et al. 2000; European Commission 2002; Kier & Kirkland 2013; US EPA 2013). The consensus among these reviews was that proper use of glyphosate and GBFs does not pose a genotoxic or carcinogenic hazard/risk with hazard indicating potential for adverse effects and risk indicating potential for adverse effects under actual conditions and amounts of exposure. As a result, glyphosate based herbicides have been approved for use in over 160 countries. The recent IARC conclusion was therefore inconsistent with these other reviews. Consequently, the Monsanto Company commissioned Intertek Scientific & Regulatory Consultancy to assemble a panel of experts to conduct a thorough review in the four areas considered by IARC including mechanistic data (focused on genotoxicity and oxidative stress). This review section reports the views of the Expert Panel of genetic toxicologists on the genotoxicity of glyphosate, GBFs and AMPA and discusses how they relate to the IARC opinions. The views and conclusions represent those of the Expert Panel of genetic toxicologists as independent scientific consultants and neither employees of the Monsanto Company nor attorneys reviewed this manuscript prior to submission.

Proper methods to accurately evaluate and interpret complex sets of genetic toxicology data

Characteristics of genetic toxicology tests and genetic testing data sets

Due to interest in understanding the potential to produce adverse effects, chemicals such as glyphosate, for which there is widespread human exposure, are typically subjected to extensive testing for genotoxic activity. The resultant database will contain studies that encompass diverse phylogenetic boundaries, types of genetic alterations, and exposure methods. Some of the more common test methods are often represented by multiple entries in the database. Proper evaluation of such data sets requires an approach that is both systematic and critical.

In large datasets, there are always likely to be some positive responses that are described as "false" or "misleading" positives from the standpoint of predicting carcinogenicity or relevance to carcinogenic mechanism (Waters et al. 1988; Mendelsohn et al. 1992; Jackson et al. 1993). False or misleading responses generally fall into one of three types:

1. Non-predictive – positive responses produced by non-carcinogenic agents. It is well documented that misleading positive responses are more frequent in certain genotoxicity tests (particularly in *in vitro* mammalian cells) due to their inherent lack of specificity (Kirkland et al. 2005; Pfuhler et al. 2011; Walmsley & Billinton 2011) and artifacts resulting from *in vitro* treatment conditions (Halliwell 2003).

2. Secondary response – the positive response is not associated with direct DNA-reactivity of the agent or metabolites of the agent but is a downstream or indirect consequence of high levels of cytotoxicity (Kirsch-Volders et al. 2003; Pratt & Barron 2003) or extreme treatment conditions such as high osmotic conditions or significant variations in pH (Scott et al. 1991). Such responses may not be relevant to *in vivo* prediction because they involve effects generated by exposures that exceed potential *in vivo* exposures.
3. Technical deficiencies – positive responses may be produced by inadequate study designs, mistakes made during the conduct of a test or inappropriate evaluation of data. This type includes cases where there is reason to question whether a positive experimental result has actually been obtained.

An understanding of possible actions leading to false or misleading responses with respect to carcinogenicity prediction or carcinogenic mechanism must be incorporated into the design, conduct, evaluation, and interpretation of genotoxicity assays. As a consequence, new standard test guidelines for *in vitro* mammalian assays published by the Organization for Economic Cooperation and Development (OECD) and other organizations indicate that treatment conditions must be monitored for maintenance of normal physiological parameters.

Therefore, it is expected that a chemical as heavily tested as glyphosate would exhibit some positive responses in its genotoxicity database that would be considered "misleading" and therefore not predictive of its true genotoxic or carcinogenic hazard/risk potential.

Methods applicable to evaluation and interpretation of complex data sets

The universally recommended method for evaluating the databases of the type associated with glyphosate (including GBFs and AMPA), involves the application of a WoE approach as discussed recently for genetic toxicology testing (US FDA 2006; Dearfield et al. 2011). Many of the principles of the WoE analysis indicated here are consistent with and included in the very recently issued endpoint specific guidance document of the European Chemicals Agency (ECHA 2015).

While numerous attempts to develop a standard WoE method to evaluate large, complex data sets have not found universal acceptance, some critical performance requirements for WoE approaches have been identified by the US EPA (Suter & Cormier 2011). One of the most important requirements is that individual test methods should be assigned a weight that is consistent with their contribution to the overall evidence, and different types of evidence or evidence categories must be weighted before they are combined into a WoE.

The weight of a category of evidence used in the Expert Panel evaluation is based on four considerations:

1. **Different categories of evidence (i.e. assay types) have different weights**. Genotoxicity tests measuring mutations and chromosome damage have greater weight than "indicator" assays that measure DNA damage. For example, for human pharmaceuticals, ICH S2 (R1) (ICH 2011) states that "fixation of damage to DNA in the form of gene mutations, larger scale chromosomal damage or recombination is generally considered to be essential for heritable effects and in the multi-step process of malignancy". The following comments are taken from the "Overview of the Set of OECD Genetic Toxicology Test Guidelines and Updates Performed in 2014–2015" (OECD 2015): *"There are tests that detect primary DNA damage (i.e. the first in the chain of events leading to a mutation), but not the consequences of this genetic damage. The endpoint measured in these tests does not always lead to a mutation, a change that can be passed on to subsequent generations (of cells or organisms). The DNA damage measured in the comet assay, or the unscheduled DNA synthesis (UDS) test, may lead to cell death, or it may initiate DNA repair, which can return the DNA either to its original state or result in mutation. When evaluating the mutagenic potential of a test chemical, more weight should be given to the measurement of permanent DNA changes (i.e. mutations) than to DNA damage events that are reversible."*
2. **The aggregate strength (robustness of protocols and reproducibility) and quality of evidence in the category also influence the weight.** It is generally acknowledged that studies conducted in compliance with Good Laboratory Practice (GLP) Regulations and studies conducted according to OECD guidelines have greater weight than studies lacking these attributes. These are fundamental features of the Klimisch scoring system, which is widely used to assess the reliability of study data, particularly for regulatory purposes (Klimisch et al. 1997).
3. **The number of pieces of evidence within a category influences the weight.** A single (or few) divergent responses (positive or negative) within a majority of studies exhibiting concordant findings would be insufficient to alter the direction and strength of the WoE. This component of the overall WoE is an aggregate of the weights of all the pieces of evidence within a single test category (e.g. tests for gene mutation).
4. **Tests with greater ability to extrapolate results to humans carry greater weight.** Test responses able to more accurately predict potential hazard in humans, such as *in vivo* tests, will generally be weighted more heavily than evidence developed from tests conducted *in vitro* or in non-mammalian models.

Human versus non-human test results

Using a variety of different methods, genotoxicity test data can be derived from human populations exposed under typical use conditions. Human population monitoring studies, if performed with sufficient sample sizes, knowledge of exposure levels and adjusted appropriately for confounding variables, can offer highly relevant information. Poorly controlled human biomonitoring studies, however, can lead to erroneous conclusions (Schmid & Speit 2007; Dusinska & Collins

2008). Adjustments that need to be considered in human biomonitoring studies for genotoxicity must extend beyond age, gender, smoking, alcohol, tobacco use, and medicines used. Diet, disease status (e.g. presence of inflammatory diseases), seasonal variation, and physical stress are all important confounding factors that influence an individual's background level for any parameter under consideration (Moller 2005; Battershill et al. 2008; Bonassi et al. 2011; Fenech et al. 2011; Tenorio et al. 2013; Collins et al. 2014). There is evidence that different factors may have different impact depending on the specific genotoxic endpoints (e.g. Fenech et al. 2011 for the cytokinesis block MN endpoint; Collins et al. 2014 for the comet endpoint).

It is worth noting that there is currently considerable debate concerning the relevance of increased levels of micronuclei in human biomonitoring studies. Speit (2013) suggested that micronuclei induced in the cytochalasin B micronucleus assay used in human biomonitoring studies, do not represent micronuclei that were induced during exposure, but rather represent DNA damage that generates micronuclei during the *in vitro* culturing required for the assay. As such, this bioassay could be classified as an "indicator test" of DNA damage with lower relevance for genotoxic risk. Kirsch-Volders et al. (2014), however, considered gaps in the knowledge regarding the source of micronuclei observed in human biomonitoring studies, but considers the assay, especially with modifications, to have utility for human genotoxic hazard/risk measurements. For the purposes of this review, the Expert Panel adopted a conservative approach and the measurement of micronuclei detected in studies of exposed humans was assigned a high weight.

It is also possible to conduct genetic tests using human derived cell lines or in primary lymphocyte cultures. With respect to results from cell lines of different origin, the benefits of using human rather than rodent derived cell lines are not as compelling as one might presume. Cell lines (human or rodent origin) with mutations affecting how cells handle initial DNA damage (e.g. p53 mutations) are typically more susceptible to genetic damage. Consequently, human cell lines with altered responsiveness to DNA damaging mechanisms may be expected to generate results not dissimilar to those produced in rodent cell lines. At this time there are not enough data available to reliably determine if the use of p53-competent cell lines of human origin (as opposed to p53-competent rodent derived lines) or other human cells confer greater accuracy (Walmsley & Billinton 2011; Fowler et al. 2014).

The most current OECD *in vitro* mammalian cell chromosomal aberration and micronucleus test guidelines indicate that either human or rodent cell lines or primary cultures may be used (OECD 2014a, 2014d). These guidelines also state that: *"At the present time, the available data do not allow firm recommendations to be made but suggest it is important, when evaluating chemical hazards to consider the p53 status, genetic (karyotype) stability, DNA repair capacity and origin (rodent* versus *human) of the cells chosen for testing."*

Thus, any *in vitro* mammalian cell results should be interpreted with caution, and the weight they contribute to an overall assessment of genotoxic activity should take account of the potential limitations.

A summary of assumptions, results, and conclusions regarding the IARC genotoxicity evaluation of glyphosate, GBFs, and AMPA

The Expert Panel used the considerations discussed above when assigning weights to genotoxicity endpoints and to the responses present in the glyphosate (and related materials) dataset. The results of this review indicate some areas of agreement with IARC, but also identified some major differences between the conclusions of the two assessments.

An evaluation of IARC and expert panel review processes

The Expert Panel agreed that there was sufficient evidence to conclude that glyphosate and GBFs appeared to induce DNA strand breaks and possibly micronuclei in *in vitro* mammalian and non-mammalian systems and sister chromatid exchanges (SCEs) in *in vitro* mammalian systems. These results provide some evidence of genotoxicity, but it is not possible to accurately characterize or classify genotoxic hazard/risk or carcinogenesis mechanisms based on these results alone. As noted earlier and further stated in the OECD overview comments (OECD 2015) regarding test weights, "*When evaluating the mutagenic potential of a test chemical, more weight should be given to the measurement of permanent DNA changes (i.e. mutations) than to DNA damage events that are reversible.*" Consequently, positive responses in genotoxic endpoints identified above as "indicator tests" (i.e. DNA strand breaks, SCEs) are evidence of compound exposure but not sufficient to determine compound effect. In order to determine compound effect, consideration must be given to available evidence clearly demonstrating the induction of gene mutations or stable chromosomal alterations, particularly *in vivo* in mammalian systems.

Evidence weighting

Weights assigned to individual assays represent the strength of evidence assigned to an endpoint or category and may be derived from validation studies supporting the endpoint's involvement in carcinogen prediction as well as its relevance to mechanisms involved with initiation of malignancy (ICH 2011). In general human and *in vivo* mammalian systems have the highest test system weight, with a lower degree of weighting applied to *in vitro* mammalian cell systems and *in vivo* non-mammalian systems and lowest weight to *in vitro* non-mammalian systems (with the exception of the well validated bacterial reverse mutation "Ames" tests using mammalian metabolic activation). Other considerations, such as response reproducibility or GLP compliance, may influence the weight of a particular study result. GLP compliance indicates a high degree of, and standard for, detailed documentation of experimental conditions and data.

Section 4.2.1 of the IARC Monograph does not provide sufficient information to its readers regarding the strategy

employed by IARC reviewers in assessing the WoE; therefore, it is not possible to know if, for example, studies were assigned variable weights in accordance with the criteria discussed above. While the Expert Panel agrees that data from a well conducted human population biomonitoring study might carry more weight in a WoE assessment, it appears that IARC considered *in vitro* studies in human cells as carrying more weight than rodent *in vivo* studies as evidenced by the order of discussion topics in Section 4.2.1, and the inclusion of a separate table for human *in vitro* studies. The overall IARC Monograph evaluation (Section 6.0) and rationale (Section 6.4) indicate that the conclusion of strong evidence of genotoxicity is based on "studies in humans *in vitro* and studies in experimental animals." As discussed above, the Expert Panel evaluation considered *in vitro* studies using cells of human origin to be weighted as equivalent to any other *in vitro* mammalian cell assay using the same endpoint.

There did not, however, appear to be additional weight assigned by IARC to other criteria such as relevance of the endpoint to neoplastic initiation, quality of study performance, *in vitro* versus *in vivo* or reproducibility of responses.

Table 1 summarizes the Expert Panel's endpoint weighting assumptions. Weights represent strength, relevance and reliability of evidence and are based on a compilation of information regarding the endpoint's reversibility and susceptibility to false or misleading positive responses with respect to carcinogenicity prediction or relevance to mechanisms involved in initiation of malignancy (Solomon et al. 1991; Pierotti et al. 2003; Petkov et al. 2015).

The endpoint and test system weighting categories are defined as follows:

- **Negligible weight** – the endpoint is not linked to any adverse effect relevant to genetic or carcinogenic hazard/risk and as such is not given weight as evidence of genotoxicity.
- **Low weight** – the end point is indicative of primary DNA damage, is not unequivocally linked to mechanisms of tumorigenicity, and the test system has low specificity.
- **Moderate weight** – the endpoint is potentially relevant to tumorigenicity or may be subject to secondary, threshold-dependent mechanisms of induction (e.g. cytotoxic clastogens, aneugens) or the test system exhibits a high rate of misleading positives with respect to carcinogenicity predictivity or carcinogenic mechanism.
- **High weight** – the endpoint is one that has been demonstrated with a high level of confidence to play a critical role in the process of tumorigenicity.

Chemical structure and chemistry of GBFs

Chemical structures of glyphosate and AMPA are presented in Figure 1. IARC did not consider the chemical structure of glyphosate in its mechanistic section; however, IARC Monograph Section 5.3 states that glyphosate is not electrophilic. Many guidelines recommend that the presence of structural alerts be considered in evaluation of or testing for genotoxicity (Cimino 2006; Eastmond et al. 2009; EFSA 2011; ICH 2011). As reported in Kier and Kirkland (2013) analysis of the glyphosate structure by DEREK software identified no structural alerts for chromosomal damage, genotoxicity, mutagenicity, or carcinogenicity. Analysis of structural alerts for genotoxicity inherently includes consideration of potential

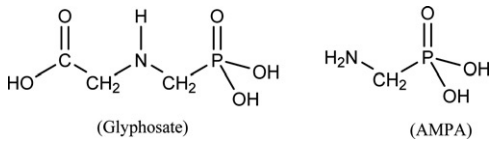

Figure 1. Chemical structures of glyphosate and AMPA. Glyphosate: N-(phosphonomethyl)glycine, acid form, CAS 1071-83-6; AMPA: aminomethylphosphonic acid; CAS 1066-51-9.

Table 1. Expert Panel's evidence weighting assumptions for mammalian (plus selected microbial test) endpoints.

Endpoint*	Negligible weight	Low weight	Moderate weight	High weight
DNA binding (adduct formation) *in vitro*		■		
DNA binding (adduct formation) *in vivo*		■		
SSB/DSB *in vitro (including comet)*		■		
SSB/DSB *in vivo (including comet)*			■	
SCEs *in vitro*	■			
SCEs *in vivo*	■			
Oxidative DNA		■		
Damage *in vitro*		■		
Oxidative DNA			■	
Damage *in vivo* (detection of 8-OHdG adducts)			■	
DNA repair effects *in vitro*		■		
DNA repair effects *in vivo*			■	
Micronuclei *in vitro*			■	
Micronuclei *in vivo*				■
Chromosomal aberrations *in vitro*			■	
Chromosomal aberrations *in vivo*				■
Gene mutation in bacteria (Ames Test)				■
Gene mutation mammalian *in vitro*			■	
Gene mutation *in vivo*				■

*Shaded box indicates weight for the endpoint. SSB: single strand breaks; DSB: double strand breaks; SCE: sister chromatid exchange.

metabolites. Although formal analysis is not available, it does not appear likely that the metabolite AMPA (glyphosate without a carboxymethyl group) has structural alerts. While structural alerts are not as definitive as experimental data, they serve as part of a WoE (Dearfield et al. 2011). The lack of structural alerts in the glyphosate molecular structure suggests lack of genotoxicity or that genotoxic effects might well be secondary to toxicity or resulting from mechanisms other than DNA-reactivity.

Another aspect of chemistry that should be recognized is the fact that GBFs, while containing glyphosate (often present as a sodium or potassium salt) also contain other components which frequently include surfactants. Specific formulations differ in composition and differences may exist between GBFs identified with a common brand name. Frequently, GBFs are observed to have greater toxicities than glyphosate. Evaluation of genotoxicity results for glyphosate and GBFs should always consider the possibility that effects observed with GBFs may be due to GBF components other than glyphosate and that there may be chemical differences between various GBFs.

The case for including other published results in the IARC genotoxicity evaluation

Although IARC policies and Working Group decisions excluded consideration of additional data from unpublished studies or publicly unavailable governmental reports, it was the Expert Panel's conclusion that the genetic toxicology studies published in reviews such as Kier and Kirkland (2013), in particular the supplementary primary data submitted with the paper, should have been considered by IARC in evaluating the genetic toxicology of glyphosate and GBFs. Though the primary study reports from which the data were extracted were not available to IARC, detailed data were provided in the Kier and Kirkland (2013) review and exceed the weight of data in most published reports that were considered by IARC. Regulatory studies of GBFs and AMPA summarized in Williams et al. (2000) should also have been considered and information on these studies is presented in Appendices A and B.

Inclusion of the studies in these publications would have filled data gaps, supplemented study categories for which there were limited numbers of test responses and would have added a very high level of confirmation to other core assay results. Table 2 summarizes an additional 90 studies covering a range of test categories that were available for review if the regulatory studies in the Kier and Kirkland (2013) publication and other published or publicly available studies had been included. Among the 90 studies not included in the IARC Monograph, only nine were reported as positive. Inclusion of these studies in a WoE produces a much clearer, more reliable and balanced assessment of the genotoxicity of glyphosate, GBFs and AMPA.

The rationale supporting the inclusion of these 90 additional studies is that the supplementary tables presented in the Kier and Kirkland (2013) paper, and presented in Supplemental Information, Appendix A of this publication, do contain sufficient detail concerning the robustness of the studies. For the regulatory studies, which were the key studies not reviewed by IARC, the Kier and Kirkland (2013) paper clearly states:

> Each study examined was stated to have been conducted in accordance with GLP standards with almost all studies citing the OECD Principles of Good Laboratory Practice (OECD GLP 1982, 1997). Reports also cited compliance with various national and regional GLP Guidelines (e.g. European Commission GLP Directives 87/18/EEC or 88/320/EEC; U.S. Environmental Protection Agency GLP Standards, 40 CFR Part 160; Japanese Ministry of Agriculture, Forestry, and Fisheries (MAFF) GLP Standards, 11 Nousan No. 6283). Variations from GLPs were considered not to have significantly impacted the study results.
>
> Almost all of the studies were reported to have been conducted in accordance with the relevant OECD test guidelines applicable at the time of the study. Study reports were examined to determine that the protocols and experimental methods for the report were consistent with the OECD guidelines and any deviations were noted and considered. Report data were examined to confirm the conclusion of the report regarding whether treatment-related activity had been observed.

Thus, the methods used were generally as specified in OECD guidelines, or any deviations were noted. Moreover, the studies were performed under GLP conditions, which would ensure protocol compliance and high quality data. The key aspects of each test method were detailed in the first few pages of the supplementary material in Kier and Kirkland (2013) so it is easy to see how top concentrations were chosen, what measures of cytotoxicity were used, how many cells were scored etc. Links to the guidelines were provided.

The rationale given by IARC for not including the regulatory studies in Kier and Kirkland (2013) was that the primary study reports were not available, and that the information provided in the supplementary tables was insufficient regarding topics such as details of statistical methods, choice of

Table 2. Summary of test categories, number of studies, and study responses available from Kier and Kirkland (2013) and other publically available studies not included in the IARC Monograph (details for all studies provided in Supplemental Information, Appendix A).

Test category	Endpoint	Glyphosate (Pos/Neg)	GBFs (Pos/Neg)	AMPA (Pos/Neg)	Total (Pos/Neg)
Non-mammalian (Bacterial Reverse Mutation)	Gene mutation	0/19	0/20	0/1	0/40
Mammalian *In Vitro*	Gene mutation	0/2	ND	ND	0/2
	Chromosomal aberrations	1/5	1/0	ND	2/5
	Micronucleus	2/0*	1/0	ND	3/0
	UDS	0/1	ND	0/1	0/2
	SCE	ND	1/0	ND	1/0
Mammalian *In Vivo*	Chromosomal aberrations	0/1	2/0*	ND	2/1
	Micronucleus	0/13*	0/17	0/1	0/31
	SCE	ND	1/0	ND	1/0
Total		3/41	6/37	0/3	9/81

*Inconclusive studies not included in count; AMPA: aminomethylphosphonic acid; GBFs: glyphosate based formulations; ND: not done.

highest dose tested, and verification of the target tissue exposure.

This rationale for exclusion is unjustified for the following reasons.

For bacterial reverse mutation assays the concentrations tested were detailed in every table, as were critical aspects of the methods (e.g. plate incorporation or pre-incubation for the Ames tests, inducing agent for the S9 and its final concentration, and number of replicate cultures). Thus, it is clear what top concentrations were used, whether they complied with the maximum concentration/dose as recommended in OECD guidelines, or whether they were defined by toxicity.

Almost all of the many Ames tests on glyphosate used a top concentration of the maximum required, 5000 μg/plate unless contraindicated by toxicity. All of the required strains, including either TA102 or *Escherichia coli,* have been used in the regulatory studies included in Kier and Kirkland (2013). The Ames tests on GBFs used quite variable top concentrations. Some went as high as the maximum required (5000 μg/plate) but others only reached <100 μg/plate, seemingly limited by toxicity. Since we know glyphosate *per se* is not very toxic in the bacterial tests, the toxicity is presumably caused by the other components of the formulations, which were more toxic in some GBFs than in others.

The mammalian cell assays on glyphosate generally reached top concentrations in the range 500–5000 μg/mL, even when prolonged (48 h) treatments were performed in the chromosomal aberration studies. Thus, many of these studies exceeded 10 mM (1690 μg/mL for glyphosate), the top concentration currently recommended in OECD guidelines for nontoxic substances. There were no regulatory mammalian cell tests on GBFs.

All except one of the regulatory *in vivo* micronucleus (MN) tests on glyphosate that used oral dosing achieved a top dose of at least 2000 mg/kg, which is the top dose for a nontoxic substance recommended in OECD guidelines. One oral study achieved a top dose of only 30 mg/kg, seemingly because severe toxicity and lethality was seen at higher doses. It is unclear why such lethal effects were seen in this study when much higher doses were tolerated in other studies using the same acute dosing regimen. Several studies using intraperitoneal (i.p.) injection had lower top doses because of greater toxicity when using the intraperitoneal route. Thus, all of the regulatory MN studies on glyphosate met or exceeded the required top dose.

The *in vivo* bone marrow MN and chromosomal aberration regulatory studies of Kier and Kirkland (2013) generally did not report evidence of target organ toxicity (e.g. %PCE, which would be a measure of bone marrow toxicity) or include analyses to demonstrate presence of glyphosate in plasma. Therefore, the issue of whether the bone marrow was exposed needs verification by evidence other than target organ toxicity.

The IARC Monograph states that about 1/3 of glyphosate administered orally to rodents is absorbed and excreted, largely unchanged, in urine. This provides evidence that it is likely that the bone marrow, a well-perfused tissue, is exposed to glyphosate in rodents treated orally. Definitive evidence of absorption and systemic distribution of glyphosate in rodents is also contained in a summary of regulatory toxicokinetic studies (JMPR 2006). These studies demonstrated absorption of glyphosate and systemic distribution, including distribution in bone marrow, in rats dosed intraperitoneally or orally. Published reports have also indicated absorption and systemic distribution of glyphosate administered by the intravenous (i.v.) or oral route in rats (Brewster et al. 1991; Anadon et al. 2009) and by the oral (dietary) route in mice (Chan & Mahler 1992). Thus, in the regulatory rodent *in vivo* MN and chromosomal aberration tests, target organ exposure would have been achieved.

If statistical analysis was performed (not commonly performed or required for Ames tests) this is given as a footnote to the supplementary tables (Kier & Kirkland 2013, supplementary tables; Appendix B, this report), together with the statistical method used, and whether the results were significant.

Thus, in view of the Expert Panel, the exclusion of these studies was not justified. Failure to evaluate and consider the large number of results included in the publication by Kier and Kirkland (2013) as well as other publicly available studies not reviewed by IARC, resulted in an inaccurate assessment of glyphosate, GBFs and AMPA's genotoxic hazard/risk potential.

Expert panel's critique of selected studies: impact on IARC evaluation

Genetic toxicology tests relied upon by most regulatory bodies to support decisions focus on a set of core endpoints that are known to be involved either in direct activation of genes responsible for neoplastic initiation in somatic cells or alteration of the genetic information in germ cells (EFSA 2011; ICH 2011; Kirkland et al. 2011). Therefore, the endpoints given the greatest weight in Table 1 include gene mutation and chromosomal aberrations.

MN formation *in vivo* was also assigned a high weight (Table 1), as it is considered an indication of chromosome breakage but could also result from aneuploidy (Kirsch-Volders et al. 2003). However, aneugenic effects are usually thresholded (Parry et al. 1994). For instance, MN may be induced by alterations in normal mitosis produced by various kinases. It was demonstrated that GBFs activate mitotic kinase CDK-1 (Marc et al. 2002) which could possibly play a role in MN induction through a separate mechanism believed to be threshold based (Terasawa et al. 2014). Although a thresholded mechanism may be considered of less weight than a non-thresholded mechanism, most *in vivo* MN studies did not investigate this. In the absence of information on clastogenic or aneugenic mode of action, the panel considered that a high weight should be applied to all *in vivo* MN studies.

Human genotoxicity biomonitoring studies

The results provided for GBFs in Table 4.1 (human studies) of the IARC Monograph concluded positive evidence of DNA breakage as determined by results in humans using the comet assay Paz-y-Miño et al. (2007), negative induction of

chromosomal aberrations (Paz-y-Miño et al. 2011), and positive induction of MN (Bolognesi et al. 2009). Due to the importance of these studies in the IARC review, these papers were critically reviewed by the Expert Panel as described in detail below.

Paz-y-Miño et al. (2007) reported increased DNA damage (comet assay) in individuals recently exposed to GBF spraying, but only "suggested" this implied a genotoxic risk. The comet assay, as discussed earlier is an "indicator" endpoint and primary DNA damage does not accumulate, so the consequences of the observed DNA breaks remain unknown (Faust et al. 2004).

The Expert Panel review of this study identified a number of issues that questioned the validity of the interpretation of results. For example, it is not clear which blood cells were scored for comets, or if it was all cells in the blood. Also, the observation of a median comet tail length of exactly 25.0 µm for 20/21 unexposed control individuals in this publication questions the quality of data collection. This unusual observation was not noted in the IARC Monograph. The Paz-y-Miño et al. (2007) publication indicated that signs of clinical toxicity were reported in the population and that the GBF application rate was reported to be some 20 times higher than recommended. The clinical signs were consistent with acute intoxication associated with severe exposures (Menkes et al. 1991) and these factors suggest that comet effects might have been secondary to toxicity from very high exposure to GBF. The Paz-y-Miño et al. (2007) report seems to qualify the conclusiveness of the results by indicating that the results "suggest" a genotoxic effect. Due to uncertainties regarding the negative control data, and particularly because of uncertainties regarding the mechanistic role of cytotoxicity in generating the effects the Panel regarded this study as inconclusive evidence for *in vivo* human genotoxic effects relevant to induction of mutations or carcinogenesis.

In a follow-up study, Paz-y-Miño et al. (2011) reported negative results for induction of chromosomal changes in individuals from areas where GBF spraying had occurred two years previously. The absence of chromosomal aberrations supports the presumption that the DNA strand breaks identified in the Paz-y-Miño et al. (2007) study were either repaired or lethal and did not persist as lesions which could be expressed as chromosomal aberrations in cultured lymphocytes in the follow-up study.

Bolognesi et al. (2009) reported a significant but small, transient and inconsistent effect of glyphosate spraying on MN induction in individuals living in areas where aerial spray application of glyphosate occurred (Figure 1 in Bolognesi et al. 2009), but concluded that any risk was "low". Of greater importance however, is the observation that no statistically significant increase in the frequency of micronucleated binucleated cells (BNMN) was observed in individuals that actually reported direct exposure to the spray compared to individuals who lived in the spray area but were not present during spraying (Bolognesi et al. 2009, Table 4). These results are shown graphically in Figure 2 (graph provided by K. Solomon). As indicated in Table 4 of Bolognesi et al. (2009), statistical analysis did not indicate a significant difference ($p < .05$, ANOVA) in post-spray BNMN frequency between

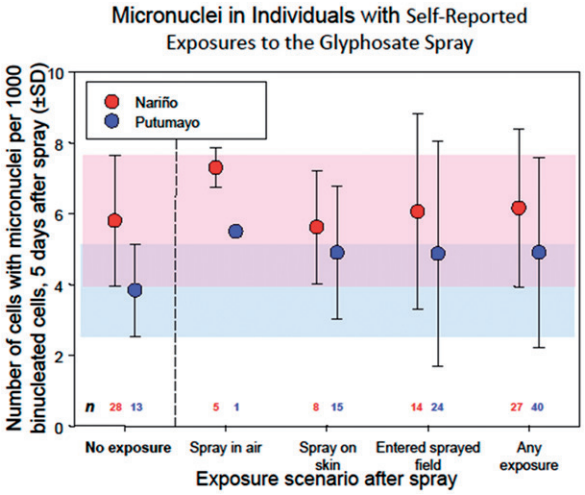

Figure 2. Mean frequency of binucleated cells with micronuclei (BNMN) in self-reported exposures to glyphosate spray in areas where aerial application occurred. From Bolognesi et al. (2009); Table 4. Data from Valle del Cauca not shown in graph since only one individual reported exposure. Graph provided by K. Solomon.

different categories of self-reported spray exposure and there was no statistically significant difference ($p < .05$) between no exposure and any self-reported spray exposure for any of the three regions. The Valle del Cauca region, which exhibited the highest post-spraying increase, only had 1/26 persons self-reporting spray exposure and the GBF spray application rate was substantially lower than the application rates in the other two regions.

Although results were temporally consistent with GBF spraying, the lack of significant correlation between increased post-spraying BNMN frequencies and self-reported spray exposure, and inconsistency with application rates, indicate that the MN effects observed in this study cannot be associated with GBF exposure (Figure 2) and therefore the Expert Panel concluded the results to be negative. The panel agrees with the statement made in the discussion section of Bolognesi et al. (2009) that based on the Bradford Hill criteria (Hill 1965) it is not possible to assign causality to the BNMN increases observed in their study and notes that elsewhere in this publication the authors seemed to qualify their conclusions with terms like "suggest" and "potentially". Lack of clear evidence of causality indicates that it is inappropriate to conclude that GBF induces MN in humans. The Bolognesi et al. (2009) results were considered negative by the Expert Panel because there were no statistically significant increases in MN frequency associated with self-reported spray exposure. This conclusion is subject to the limitation of the use of self-reporting as a measure of exposure.

The Expert Panel conclusion for the Bolognesi et al. (2009) results seems to be quite different from the IARC Monograph. The qualifications about lack of consistency with exposure rates or statistically significant association with self-reported spray exposure are noted in the discussion of this study in IARC Monograph Section 4.2.1(a)(i). However, these qualifications are not evident in IARC Monograph Section 5.4 which presents these results as positive without qualification. IARC Monograph Section 6.4 not only presents the results as

positive without qualification but seems to give this study a high weight in arriving at their conclusion of a genotoxic mode of action.

Due to the deficiencies cited in the biomonitoring studies above, along with the lack of scientific consensus regarding the relevance of MN found in exposed humans, the Expert Panel concluded that there was little or no reliable evidence produced in these studies that would support a conclusion that GBFs, at levels experienced across a broad range of end-user exposures, poses any human genotoxic hazard/risk.

Studies in mammalian in vitro and in vivo assays

The number of studies conducted in mammalian models both *in vitro* and *in vivo* was relatively extensive but with some notable data deficiencies and gaps. However, looking for evidence consistent with a concern for genotoxic hazard finds little or no compelling support among test methods that assess relevant endpoints.

Gene mutation

IARC noted one negative *in vitro* mammalian gene mutation result for glyphosate (IARC Monograph Table 4.4). Additionally there are two negative results for glyphosate in the mouse lymphoma tk locus assay (Kier & Kirkland 2013). These provide a clear WoE that glyphosate does not induce gene mutation in mammalian cell systems. There are no *in vitro* mammalian cell gene mutation results for GBFs or AMPA.

Chromosomal effects in vitro

In *in vitro* mammalian cell chromosomal aberration assays (IARC Monograph Tables 4.2 and 4.4) glyphosate was reported positive in one study and negative in two other studies. Regulatory studies and published studies, not considered by IARC, provide one additional positive result and five additional negative results (see Supplemental Information, Appendix A, Table 2 of this paper). One of the positive studies (Lioi et al. 1998a) is not considered valid due to the fact that there was excessive cytotoxicity (>50% reductions in mitotic index at all concentrations tested, exceeding current regulatory guidelines for a valid assay). Several of the published studies did not include exogenous mammalian metabolic activation. Most importantly, the negative studies tested glyphosate at dose levels well in excess of those reported positive by Lioi et al. (1998a, 1998b) and included several human and bovine lymphocyte studies. In addition to the negative chromosomal aberration assays the two negative results in the mouse lymphoma tk locus assay also add weight to a conclusion that glyphosate is not clastogenic in *in vitro* mammalian cell assays. Overall these results provide sufficient evidence that glyphosate is not clastogenic in mammalian cells when studied under appropriate *in vitro* treatment conditions.

No *in vitro* mammalian chromosomal aberration studies of GBFs and one positive *in vitro* mammalian chromosomal aberration study with AMPA were reported by IARC. The latter study by Sivikova and Dianovsky (2006), reported as a GBF study in IARC, is considered to be a study of a manufacturing batch of an isopropyl salt of glyphosate from a Monsanto source (Kier & Kirkland 2013). An additional positive *in vitro* mammalian chromosomal aberration study was not considered by IARC (Amer et al. 2006; see Supplemental Information, Appendix A, Table 2 of this paper). The positive GBF study tested an unusual GBF and employed very high dose levels. These single studies do not provide a strong WoE for induction of chromosomal aberrations for GBFs or AMPA in mammalian cells *in vitro*.

IARC reported two positive *in vitro* mammalian cell MN studies of glyphosate. However, another four positive or equivocal *in vitro* mammalian cell MN studies of glyphosate were identified in the literature that were not reported in IARC but were summarized in Kier and Kirkland (2013). Several of the studies had weak or inconsistent responses. Piesova (2004, 2005), not in IARC, reported statistically significant increases in MN in bovine lymphocytes only with 48-h incubation without S9 metabolic activation but the responses were not consistent between donors. Two papers by Mladinic et al. (2009a, 2009b) reported weak responses in human lymphocytes at the highest dose tested in the presence of S9 metabolic activation. MN results for Mladinic et al. (2009a) were not reported in IARC. One of these studies (Mladinic et al. 2009a) had a very high control MN frequency and in both publications it appears that cells were treated prior to mitogen stimulation which would mean cells would have been exposed in G0 cell stage. This treatment regimen is not considered appropriate according to current test guidelines. The MN induced at high doses were predominantly centromere positive suggesting the possibility of an aneugenic effect. These responses were considered of limited quality by IARC and the publication authors indicated that the high dose effects might have been at a dose level exceeding a threshold and possibly associated with high toxicity. Koller et al. (2012), MN results not evaluated by IARC, reported positive *in vitro* MN results in human-derived buccal epithelial cells for glyphosate in the absence of S9 metabolic activation. An unusual feature of this paper was indication of significant cytotoxicity at very low dose levels (20 μg/mL) and with very short exposure times (20 min). Although the authors speculated their epithelial cells might be more sensitive than cells of the hematopoietic system such as lymphocytes, a large number of other studies using non-hematopoietic cells used much higher doses and longer exposure times. A study by Roustan et al. (2014) reported increases in MN frequency in CHO-K1 cells only in the presence of S9 activation. There was very little dose response observed over an order of magnitude of concentrations (10–100 μg/mL). Thus, although positive (or equivocally positive) responses were observed for glyphosate in several studies these responses were not consistent in terms of dose levels or requirement for an S9 metabolic activation system. The possibility of a threshold aneugenic effect in the presence of S9 metabolic activation might be suggested by the results of Mladinic et al. (2009a, 2009b) but other studies cannot confirm this possibility because presence or absence of centromeres was not

measured. It should be noted that there is a report that glyphosate is essentially unchanged by incubation with rat liver homogenate which would indicate that S9 activation dependent responses might not be due to metabolites of glyphosate (Gohre et al. 1987).

Overall these studies provide only very limited evidence of the possibility of MN induction by glyphosate in *in vitro* mammalian cell assays and this observation, coupled with the negative profile for clastogenicity in *in vitro* mammalian cell assays, would suggest this low possibility is limited to aneugenic effects that are likely to be indirect and thresholded.

Although IARC reports one negative *in vitro* mammalian cell assay with a GBF (Sivikova & Dianovsky 2006), as noted above this assay is likely to have been performed with a technical glyphosate preparation rather than a formulation. Koller et al. (2012) report a positive *in vitro* MN result for a GBF (result not included in IARC) in buccal epithelial cells derived from a human-neck metastatic tumor. The authors noted that these cells have not been used for genotoxicity assessments and the Expert Panel considered the results in this non-validated system to be of unknown relevance. IARC reported one positive result for AMPA in an *in vitro* mammalian cell MN assay in CHO-K1 cells (Roustan et al. 2014). An unusual feature of the Roustan et al. (2014) study was that AMPA apparently exhibited much higher cytotoxicity than glyphosate. Although complete cytotoxicity data are not presented, the maximum AMPA concentrations evaluated for MN, appearing to produce less than 50% reduction in cytokinesis blocked proliferation index, were 1000-fold lower than glyphosate concentrations in the absence of S9 metabolic activation, 20-fold lower in the presence of S9 metabolic activation and 100,000-fold lower with light activation. These very large cytotoxicity differences are dramatically different from the relative toxicities of AMPA and glyphosate observed in other mammalian cell studies, e.g. Chaufan et al. (2014); Manas et al. (2009a, 2009b); Li et al. (2013); Kwiatkowska et al. (2014). These individual studies, particularly the Roustan et al. (2014) study, appear to exhibit technical problems and do not present a convincing WoE for *in vitro* mammalian cell MN effects of GBFs or AMPA.

Chromosomal effects in vivo

As a general point, it was noted earlier that there is adequate evidence available from toxicology studies demonstrating absorption and distribution of glyphosate to bone marrow in the rat (i.p., i.v., and oral routes) and absorption and distribution of glyphosate in blood by the oral route in the mouse. This information provides evidence for target organ exposure in the rodent bone marrow studies discussed below, which is particularly important when negative results are obtained.

Table 4.3 in the IARC Monograph reported one negative *in vivo* rat bone marrow chromosomal aberration result and one negative mouse dominant lethal result for glyphosate. In addition there is one negative regulatory *in vivo* mouse bone marrow chromosomal aberration study of glyphosate not evaluated by IARC (Suresh 1994; see Supplemental Information, Appendix A, Table 3). These studies provide *in vivo* evidence complementing the larger number of *in vitro* studies (discussed above) indicating glyphosate is not clastogenic when tested in mammalian assays.

IARC reported two positive results and one negative result for glyphosate in *in vivo* MN assays. In one of the positive studies reported by IARC (Bolognesi et al. 1997), relatively low increases in MN frequency were observed which might well be within the historical range of many laboratories (Salamone & Mavournin 1994). The other positive study (Manas et al. 2009a) had an unusual feature in that it is reported that erythrocytes were scored for MN, but in the bone marrow and at an early sampling time. Historical control data were not reported in the publication so the relevance of this result cannot be determined. By contrast, there are an additional 13 published, publicly available or regulatory *in vivo* MN studies with glyphosate in the mouse (12 studies) or rat (one study), all of which gave negative results (see Supplemental Information, Appendix A, Table 3 of this paper). These negative results were obtained in multiple studies at dose levels that exceeded those at which positive results had been reported in the IARC reviewed studies mentioned above using the same (i.p.) route of administration. With respect to a route of exposure, the negative MN results in a glyphosate mouse feeding study (Chan & Mahler 1992) that was not reported in IARC are of particular relevance to carcinogenic potential. The Expert Panel's conclusion is that there is a strong WoE that glyphosate does not induce MN *in vivo* in mammals.

IARC reported one positive and one negative rodent bone marrow chromosomal aberration study for GBFs. An additional two published positive rodent chromosomal aberration studies on GBFs were identified that were not reported in IARC. One mouse study with positive results (Prasad et al. 2009) employed sampling times for a chromosomal aberration assay quite different from those currently recommended (OECD 2014c). Moreover, the GBF was administered i.p. using dimethylsulfoxide (DMSO) as a vehicle and the use of this vehicle and route has unusual toxicity properties (Heydens et al. 2008). This assay was also unusual in that dose-responsive increases were observed at multiple sampling times, which is difficult to explain since cells damaged at early sampling times have usually died and disappeared from the bone marrow by later sampling times. Another positive publication (Amer et al. 2006), not reported in IARC, found positive chromosomal aberration results in mouse bone marrow and spermatocytes with treatments that included repeated oral and i.p. dosing. The test material was reported to be a formulation containing 84% glyphosate which is very unusual and raises the possibility that observed effects were due to some unusual or unique component of this formulation. Another published positive GBF study (Helal & Moussa 2005) uniquely involved rabbits exposed to GBF (750 ppm) in drinking water for 60 days. Using extended repeat dosing for a bone marrow chromosomal aberration assay is questionable because cells with chromosome breaks usually do not accumulate and any cytogenetic effects would likely be due to the final one or two doses. Total aberrations reported for this study included some nonstandard and questionable categories such as gaps and centromeric attenuations. Thus, most of the positive *in vivo* chromosomal aberration studies with

GBF's are all subject to concerns regarding the reliability or biological relevance of the results. While they cannot be ignored, they do not warrant undue weight, and do not support a conclusion of strong evidence of genotoxicity.

IARC reported two positive and three negative *in vivo* rodent bone marrow MN results for GBFs. One of the two positive studies (Bolognesi et al. 1997) had low negative control MN frequencies and the MN frequencies in treated groups were within historical control ranges for many laboratories (Salamone & Mavournin 1994) although historical control ranges for the laboratory were not reported in the publication. The other positive study (Prasad et al. 2009) was unusual in using DMSO as a vehicle by the i.p. route which, as noted above, may have led to unusual toxicity. However, there are an additional 17 rodent bone marrow studies with GBFs that were not considered by IARC, and all were negative (see Supplemental Information, Appendix A, Table 3 of this paper). The negative studies included use of both oral and i.p. routes and maximum dose levels frequently were limit doses of 2000 mg/kg (OECD 2014b). The overwhelming majority of *in vivo* MN studies on GBFs, therefore, gave negative results. In the studies reported positive, there are indications that the results may not be biologically meaningful, or that artifacts may have resulted from use of DMSO as vehicle.

For AMPA, IARC reported one positive mouse bone marrow MN study. There was one negative regulatory mouse bone marrow MN study of AMPA not reported in IARC. Both studies used the i.p. route. The positive study used a top dose of 200 mg/kg administered on two occasions, 24 h apart. The negative study used a single top dose of 1000 mg/kg which produced signs of toxicity. There is no obvious explanation for these conflicting results and the limited data do not allow reasonable WoE conclusions for AMPA in terms of the *in vivo* MN endpoint.

DNA damage in vitro

As noted above, the Expert Panel is in agreement with IARC reviewers that there are several *in vitro* mammalian cell studies of glyphosate which show DNA strand break effects (more specifically the alkaline single cell gel electrophoresis or comet endpoint). However, as also noted above, these studies should be assigned low weights compared to other more relevant endpoints in evaluating genotoxic risk, particularly when the results for relevant endpoints are more abundant. An assumption that the DNA damage observed *in vitro* might be secondary to toxicity rather than leading to DNA-reactive or persistent genotoxicity is underscored by cases where the same publication reports DNA damage effects but not chromosomal alterations, e.g. Sivikova and Dianovsky (2006); Manas et al. (2009a); Mladinic et al. (2009a) without metabolic activation. Other publications reported both DNA damage and chromosomal effects, e.g. Lioi et al. (1998a); Koller et al. (2012).

For GBFs there are only two positive *in vitro* mammalian cell comet results reported by IARC. These provide limited evidence for GBF-induced DNA damage effects *in vitro* in mammalian cells.

There are a few positive *in vitro* mammalian cell SCE reports for glyphosate and GBFs reported in IARC. Since the OECD guideline for the SCE test has recently been deleted because of a lack of understanding of the mechanism(s) detected by the test, the biological relevance of SCE is unclear, and these studies have not been further considered by the Expert Panel for a WoE evaluation.

One negative primary hepatocyte UDS result is reported by IARC for glyphosate, but there are also negative primary hepatocyte UDS results for glyphosate and AMPA (one each) not reported by IARC.

DNA damage/adducts in vivo

One *in vivo* mammalian DNA damage and one *in vivo* mammalian DNA adduct study of glyphosate were reported by IARC. No additional regulatory or published studies were identified. Results for 8-hydroxydeoxyguanosine (8-OHdG) measurements are considered in the oxidative stress section (Section IIIB).

Bolognesi et al. (1997) reported transient (4 h after dosing) increases in alkali-labile DNA strand breaks in liver and kidneys of mice treated i.p. with glyphosate. Interpretation of the genotoxic significance of these observations is difficult because such effects might be due to arrest of cells in S-phase or secondary to cytotoxicity (Williams et al. 2000). Peluso et al. (1998) reported no induction of adducts in mouse liver or kidney detectable by ^{32}P-postlabelling methodology after i.p. administration of glyphosate.

There is one positive *in vivo* SCE report for a GBF by Amer et al. (2006) which was not evaluated by IARC. For reasons of relevancy noted above, this study has not been further considered by the Expert Panel in a WoE evaluation.

One *in vivo* mammalian DNA damage and one *in vivo* mammalian DNA adduct studies of GBFs were reported by IARC. No additional regulatory or published studies were identified.

Bolognesi et al. (1997) reported transient (4 h after dosing) increases in alkali-labile DNA strand breaks in liver and kidneys of mice treated i.p. with a GBF. Similar conclusions about interpretation of these results apply as for the glyphosate results by the same authors discussed above. Peluso et al. (1998) observed ^{32}P-postlabelling adducts in liver and kidneys of mice dosed with a GBF. The source or identity of the adducts were not characterized although such adducts were not observed in studies with glyphosate in their publication.

No *in vivo* mammalian DNA damage studies of AMPA were reported in IARC or identified.

The paucity of data as well as the limited significance of the primary DNA damage endpoints on tumor initiation did not warrant that these observations should have a significant WoE impact.

Weight of evidence (WoE) for genotoxic effects in mammalian systems

In summary, the WoE from *in vitro* and *in vivo* mammalian tests for genotoxicity indicates that:

- Glyphosate does not induce gene mutations *in vitro*. There are no *in vitro* mammalian cell gene mutation data for GBFs or AMPA, and no gene mutation data *in vivo*.
- Glyphosate, GBFs, and AMPA are not clastogenic *in vitro*. Glyphosate is also not clastogenic *in vivo*. Some positive *in vivo* chromosomal aberration studies with GBFs are all subject to concerns regarding their reliability or biological relevance.
- There is limited evidence that glyphosate induces MN *in vitro*. Although this could be a reflection of increased statistical power in the *in vitro* MN studies, the absence of clastogenic effects in a large majority of *in vitro* chromosomal studies suggests the possibility of threshold-mediated aneugenic effects. However, there is strong evidence that glyphosate does not induce MN *in vivo*.
- Limited studies and potential technical problems do not present convincing evidence that GBFs or AMPA induce MN *in vitro*. The overwhelming majority of *in vivo* MN studies on GBFs gave negative results, but conflicting and limited data do not allow a conclusion on *in vivo* induction of MN by AMPA.
- There is evidence that glyphosate and GBFs can induce DNA strand breaks *in vitro*, but these might be secondary to toxicity since they did not lead to chromosome breaks. There is limited evidence of transient DNA strand breakage for glyphosate and GBFs *in vivo*, but for glyphosate at least these are not associated with DNA adducts. These results are assigned a lower weight than results from other more relevant endpoints, which were in any case more abundant.
- There is evidence that glyphosate and AMPA do not induce UDS in cultured hepatocytes.
- Some reports of induction of SCE *in vitro* by glyphosate and GBFs, and one positive report of SCE induction *in vivo* by a GBF, do not contribute to the overall evaluation of genotoxic potential since the mechanism of induction and biological relevance of SCE are unclear.

Studies in non-mammalian test systems

With the exception of the bacterial reverse mutation test, global genotoxicity testing guidelines such as those issued by OECD (2015) and other regulatory bodies do not recommend routine use of non-mammalian assays. Recently, OECD guidelines for two non-mammalian tests have been deleted because mammalian cell tests are considered more biologically relevant, and non-mammalian tests (with the exception of the bacterial reverse mutation test) are rarely used for regulatory test batteries.

Table 4.6 in the IARC Monograph summarized results from two bacterial reverse mutation test publications. One publication (Li & Long 1988) reviewed by IARC reported no mutagenic activity associated with glyphosate in a bacterial reverse mutation test but a publication by Rank et al. (1993) indicated a positive finding with a glyphosate formulation.

Rank et al. (1993) reported positive mutagenicity in TA98 only without S9 and positive mutagenicity in TA100 only with S9. At the outset this combination of responses is problematic as it is an unlikely combination and suggests that either one or both strain/S9 responses would be in error. The study data shown in Table 2 of the Rank et al. (1993) publication indicates that the positive responses reported for TA98 and TA100 were neither dose related nor were they reproduced in repeat data sets. The authors called the results indicative of gene mutation capabilities for a GBF; however, the data should never have been accepted for publication without additional testing over a narrower range of doses and as they currently stand, do not meet commonly used criteria for declaring Ames test results positive. The data from this one publication are not in agreement with 19 bacterial reverse mutation assays of GBFs presented in Supplemental Information, Appendix A, Table 1 that were not included in the IARC Monograph. The Expert Panel considered the results of this study to be inconclusive.

A large number (20) of negative bacterial reverse mutation assays of GBFs are presented in Supplemental Information, Appendix A, Table 1. None of these were included in the IARC Monograph. There is also one negative regulatory study of AMPA.

In contrast to the two bacterial reverse studies considered in the IARC Monograph there are actually abundant data from 40 additional studies (Supplemental Information, Appendix A, Table 1) that glyphosate and GBFs are negative in the one genetic test for gene mutation considered overall to be the best non-mammalian predictor of mammalian carcinogenesis.

Publications in which glyphosate or GBFs have been tested for genotoxicity in a variety of non-mammalian species other than bacterial reverse mutation appear to be included in the IARC Monograph, with only a few regulatory or published studies not included. With the exception of two positive and one negative chromosomal aberration assays in plants for glyphosate, chromosomal effect assay results have mainly been published for GBFs and showed predominantly positive results for MN in fish and amphibians.

A larger number of DNA damage comet assays in fish and other non-mammalian species *in vitro* are reported as exhibiting predominantly positive results for glyphosate. Larger numbers of positive comet results are available for GBFs in fish and amphibian/reptile studies. One positive fish comet study is reported for AMPA.

Some general features of these non-mammalian tests should be noted. First, both major endpoints measured in the majority of non-mammalian tests (i.e. MN and comet) might well be secondary to toxic effects. Second, many of these tests involve exposure by immersion in or surface contact with the test material in water. This is certainly not a standard or relevant route of exposure for *in vivo* mammalian systems and may introduce route-specific unique toxicity and genotoxic effects. This is particularly a concern for GBFs which commonly contain surfactants.

As a consequence, the Expert Panel did not consider data from a majority of the non-mammalian systems and nonstandard tests with glyphosate, GBF, and AMPA to have significant weight in the overall genotoxicity evaluation, especially given the large number of standard core studies in the gene mutation and chromosomal effects categories available in mammalian systems. Rationale supporting this consideration

is the absence of internationally accepted guidelines for such non-mammalian test systems, lack of databases of acceptable negative control data or positive control responses, and no results from validation studies suggesting concordance with carcinogenicity. OECD guidelines specifically state that use of any nonstandard test requires justification along with stringent validation including establishing robust historical negative and positive control databases. Therefore, results in these tests, when conflicting with findings obtained in well validated test systems for which OECD guidelines exist, and where the biological relevance of the results can be evaluated, do not carry a significant WoE.

Critique of the classifications and mode of action (MoA) proposed in the IARC monograph for glyphosate and related agents

Genotoxicity classification and MoA

Based on the results of the WoE critique detailed above and the wealth of negative regulatory studies reviewed by Kier and Kirkland (2013) and Williams et al. (2000), the Expert Panel does not agree with IARC's conclusion that there is strong evidence for genotoxicity across the glyphosate or GBFs database. In fact the Expert Panel WoE assessment provides strong support for a ***lack*** of genotoxicity, particularly in study categories closely associated with indications of potential genetic and carcinogenic hazard.

In order to demonstrate how the evidence from all sources was used to develop the Expert Panel's WoE conclusions for glyphosate, GBFs, and AMPA, the results from all study types were compiled in Table 3. Wherever possible, positive or negative responses were assigned to the individual studies in Table 3 according to the conclusions given in the original publication or report. In a small number of studies the Expert Panel concluded that there were significant issues regarding data analysis and interpretation of results and either changed the positive call given by IARC, e.g. Bolognesi et al. (2009) or, if the impact of the issues on the overall conclusions of the study was considered inconclusive, the data from that paper were excluded from Table 3, e.g. Paz-y-Miño et al. (2007) and Rank et al. (1993).

It should also be noted that the weight indicated in this table primarily reflects the endpoint of the publication or report. As noted above, there are significant test system (experimental protocol and data interpretation) considerations for some specific studies that significantly lowered the weight of these studies independently of the endpoint measured.

An evaluation of the studies in Table 3 according to their relative contributions to a WoE produced the following results:

- Test methods identified as providing low contribution (Low Weight) to the WoE produced the highest frequency of positive responses, regardless of whether the responses were taken from the results of IARC evaluated studies alone (eight of nine) or from all studies combined (eight of 11).
- The highest frequencies of positive responses were reported for test endpoints and systems considered most likely to yield false or misleading positive results with respect to carcinogenicity prediction or carcinogenic mechanism due to their susceptibility to secondary effects. This relationship was constant regardless of whether the results were taken from IARC evaluated studies alone or all studies combined.
- The numbers of studies providing strong evidence of relevant genotoxicity (High Weight) were in the minority for both the IARC and Expert Panel evaluations, with six out of 15 studies identified as High Weight being positive for the IARC evaluation, and only eight out of 92 studies identified as High Weight being positive for all studies combined by the Expert Panel.

Contrary to IARC's conclusion that there is strong evidence of genotoxicity, the Expert Panel's WoE analysis of the complete database (or the IARC subset alone) using the weighting categories proposed in Suter and Cormier (2011) indicates that glyphosate and GBFs should not be classified as genotoxic. The panel does not agree with IARC's conclusion of moderate evidence for genotoxicity of AMPA. The data needed to make an assessment of the genetic hazard of AMPA are too limited and conflicting to reliably support such a classification.

To provide greater emphasis to the Expert Panel's WoE conclusion, Table 4 provides a comparison between a set of characteristics found in confirmed genotoxic carcinogens (Bolt et al. 2004; Petkov et al. 2015) and the genotoxic activity profiles for glyphosate, AMPA, and GBFs. There is virtually no concordance between the two sets of characteristics.

Oxidative stress classification and MoA

Oxidative stress was the second characteristic considered by IARC as operative in human carcinogens and thus supporting their classifying glyphosate as probably carcinogenic to humans. Publications investigating the relationship between oxidative DNA damage and cancer (Wu et al. 2004; Klaunig et al. 2010) have demonstrated that following exposure to oxidative stress-inducing agents, a common adaptive response induced in mammalian cells is the up-regulation of stress-response genes. The resultant toxic response is threshold dependent.

It has been shown that reactive oxygen species (ROS) are genotoxic in principle, and the question arises as to whether GBFs that increase ROS production will add to an endogenously produced background level of DNA lesions or whether compensatory mechanisms may result in non-linear dose-effects. Halliwell (2003) reported that alteration to DNA molecules triggers repair, and frequent activation may increase the general repair capacity, irrespective of the cause of the damage. Thus, repeated exposure to ROS may lead to an adaptive response, mitigating the mutagenicity of oxidative DNA lesions. Moreover, as suggested by Deferme et al. (2015) oxidative stress is not uniquely associated with a genotoxic carcinogens and simple measurements of ROS are insufficient

Table 3. Summary of Expert Panel's evaluation of human, non-human mammalian, and selected microbial genotoxicity studies from IARC Section 4.2.1 and other published sources.

Source	Test category	Endpoint	Weight	Glyphosate (Pos/Neg)	GBFs (Pos/Neg)	AMPA (Pos/Neg)	Total (Pos/Neg)
Kier and Kirkland (2013) and other published studies not included in IARC	Bacterial Reverse Mutation	Gene mutation	High	0/19	0/20	0/1	0/40
	Mammalian *In Vitro*	Gene mutation	Moderate	0/2	ND	ND	0/2
		Chromosomal aberrations	Moderate	1/5	1/0	ND	2/5
		Micronucleus	Moderate	2/0	1/0	ND	3/0
		UDS	Low	0/1	ND	0/1	0/2
		SCE	None	ND	1/0	ND	1/0
	Mammalian *In Vivo*	Chromosomal aberrations	High	0/1	2/0	ND	2/1
		Micronucleus	High	0/13	0/17	0/1	0/31
		SCE	None	ND	1/0	ND	1/0
IARC Monograph 112	Bacterial Reverse Mutation	Gene mutation	High	0/1	0/0	ND	0/1
	Mammalian *In Vitro*	Gene mutation	Moderate	0/1	ND	ND	0/1
		Chromosomal aberrations	Moderate	1/2	ND	1/0	2/2
		Micronucleus	Moderate	2/0	ND	1/0	3/0
		Comet/DNA breaks	Low	5/0	2/0	1/0	8/0
		UDS	Low	0/1	ND	ND	0/1
		SCE	None	3/0	2/0	ND	5/0
	Mammalian *In Vivo*	Chromosomal aberrations	High	0/1	1/1	ND	1/2
		Micronucleus	High	2/1	2/3	1/0	5/4
		Comet/DNA breaks	Moderate	1/0	1/0	ND	2/0
		Dominant lethal	High	0/1	ND	ND	0/1
	Human *In Vivo*	Chromosomal aberrations	High	ND	0/1	ND	0/1
		Micronucleus	High	ND	0/3	ND	0/3
	High Weight *Combined Totals*			2/37	5/45	1/2	8/84
	(IARC results only)			(2/4)	(3/5)	(1/0)	(6/9)
	Moderate Weight *Combined Totals*			7/10	3/0	2/0	12/10
	(IARC results only)			(4/3)	(1/0)	(2/0)	(7/3)
	Low Weight *Combined Totals*			5/2	2/0	1/1	8/3
	(IARC results only)			(5/1)	(2/0)	(1/0)	(8/1)

AMPA: aminomethylphosphonic acid; GBFs: glyphosate based formulations; ND: no data.
All responses based on study critiques and conclusions of Expert Panel members.
Non-mammalian responses from IARC monograph in this table did not include four positive studies measuring DNA strand breaks in bacteria and one negative Rec assay in bacteria from IARC monograph Table 4.6.

Table 4. Comparison of test response profiles from glyphosate, GBFs, and AMPA to the profile characteristics of confirmed genotoxic carcinogens.

Characteristic	Carcinogens with a proven genotoxic mode of action	Glyphosate, GBFs, AMPA study data in Section 4.2.1
Profile of test responses in genetic assays	Positive effects across multiple key predictive endpoints (i.e. gene mutation, chromosomal aberrations, aneuploidy) both *in vitro* and *in vivo*	No valid evidence for gene mutation in any test; no evidence for chromosomal aberrations in humans and equivocal findings elsewhere.
Structure activity relationships	Positive for structural alerts associated with genetic activity	No structural alerts for glyphosate or AMPA suggesting genotoxicity
DNA binding	Agent or breakdown product are typically electrophilic and exhibit direct DNA binding	No unequivocal evidence for electrophilic properties or direct DNA binding by glyphosate or AMPA
Consistency	Test results are highly reproducible both *in vitro* and *in vivo*	Conflicting and/or non-reproducible responses in the same test or test category both *in vitro* and *in vivo*
Response kinetics	Responses are dose dependent over a wide range of exposure levels	Many positive responses do not show significant dose-related increases
Susceptibility to confounding factors (e.g. cytotoxicity)	Responses are typically found at nontoxic exposure levels	Positive responses typically associated with evidence of overt toxicity

evidence supporting a genotoxic causal MoA for carcinogenicity (Arai et al. 2006).

The evidence for oxidative stress induction summarized by IARC comes from studies employing a variety of endpoints and test systems, but in the IARC Monograph the data on oxidative stress are comingled with data from other endpoints, and data on glyphosate and GBFs are also comingled. It is therefore difficult to obtain a clear picture of the oxidative stress effects.

Indirect measures of oxidative stress vs. measures of oxidative damage

In some respects, measures (endpoints) of oxidative effects can be weighted in a manner similar to that applied to measures of genotoxicity. For example, in the majority of the studies reviewed by IARC, the endpoints assessed were only indirect measures of oxidative stress, in the form of antioxidant suppressive effects, changes in endogenous levels of protective molecules or enzymes (e.g. glutathione, superoxide dismutase) or changes in ROS (e.g. H_2O_2). The experiments *in vitro* in mammalian cells produced conflicting results and some positive results were observed only at very high dose levels which could be problematic for reliable evaluation of the potential for *in vivo* oxidative stress (Halliwell 2003). Long et al. (2007) demonstrated that reactive oxygen can be produced as an artifact by chemical reactions with components of the culture media, a possibility not evaluated in the studies reviewed by IARC. Overall, IARC's assessment did not appear to consider the relative importance of different

biomarkers of oxidative stress with the exception of noting limitations of using dihydrofluorescein acetate as a marker of oxidative stress.

A more meaningful endpoint for identification of oxidative damage, particularly as it pertains to identification of a possible genotoxic mechanism of cancer, would be the identification and application of a biomarker relevant to oxidative stress-induced damage to DNA. While a number of biochemical and physiological changes in cells can be produced during oxidative stress, the most extensively studied oxidative DNA lesion produced is 8-OHdG. This adduct has been widely used as a biomarker of oxidative DNA damage, and determination of 8-OHdG levels may be useful in defining a chemical's MoA.

Oxidative damage studies evaluated in the IARC monograph

Peluso et al. (1998) reported ^{32}P-postlabelling adducts in rats treated with GBFs (but not glyphosate). The nature or source of the adducts was not identified but Williams et al. (2000) noted that the solvent system used by Peluso et al. (1998) could not detect oxidative DNA damage. Evidence for increased DNA damage in Bolognesi et al. (1997) as measured by 8-OHdG DNA adducts was both limited and contradictory. Glyphosate was reported to induce 8-OHdG adducts in liver but not kidney tissues whereas a GBF (with an equivalent level of glyphosate) was reported to induce 8-OHdG adducts in kidney but not in liver tissue. Results of the Bolognesi et al. (1997) study are contradicted by another published study (Heydens et al. 2008) that was not considered by IARC. In this study no statistically significant increases in 8-OHdG were observed in liver or kidneys of mice 24 h after treatment by i.p. injection with 600 and 900 mg/kg of a GBF of the same composition as those used by Peluso et al. (1998) and Bolognesi et al. (1997).

The only other cited mammalian study examining oxidative DNA damage was a measurement of the effect of human 8-oxoguanine DNA N-glycosylase 1 (hOGG1) on the comet endpoint in human lymphocytes exposed to glyphosate (Mladinic et al. 2009a). This study showed a small but statistically significant effect on comet tail intensity at only a low mid-dose level in the absence of an S9 metabolic activation system and at the highest dose level tested (580 μg/mL) in the presence of S9. The observation of an effect at the highest dose level only in the presence of S9 is unusual because statistically significant increases in other markers of oxidative stress were observed at the high dose levels in either the presence or absence of S9. The authors indicated that their results were not considered an unequivocal indication of the oxidative potential of glyphosate. As noted above there does not appear to be any significant *in vitro* metabolism of glyphosate with rat liver homogenate (Gohre et al. 1987).

A series of studies in eels examined oxidative DNA damage of glyphosate, GBF, and AMPA by measurement of comet endpoints with and without treatment of samples with endonucleases that cleave at sites of oxidative damage (Guilherme et al. 2012a, 2012b; Guilherme et al. 2014a, 2014b; Marques et al. 2014a, 2014b). When considering net effects of endonuclease treatment there were varied responses in different conditions, tissues, and treatments ranging from no statistically significant effect to relatively small but statistically significant effects. These studies did not provide consistent strong evidence of oxidative DNA damage in a non-mammalian system.

In addition there was a human biomonitoring study measuring blood 8-OHdG which did not indicate a statistically significant association between previous GBF exposure and high 8-OHdG levels (Koureas et al. 2014, not evaluated in IARC). There are concerns with this study, particularly the relationship between the timing of exposure and a presumably transient marker of exposure. While some other agents did show associations, the lack of a statistically significant association between 8-OHdG and past GBF exposure does not provide support for GBF-related oxidative DNA damage in humans.

Many more oxidative stress studies are available for GBFs than for glyphosate or AMPA. Unlike glyphosate, most of the GBF studies show evidence of oxidative stress suggesting that GBFs contain compounds that are likely to be toxic under some treatment conditions leading to ROS followed by normal cellular protective responses. Comparison of GBF oxidative stress study results with predicted human exposure levels (e.g. calculated 90th percentile for applicators of 0.064 mg/kg body weight/day and much lower for other exposures), suggests that it is not likely that GBFs would induce oxidative stress likely to exceed endogenous detoxification capacities.

IARC claims of strong evidence supporting oxidative stress from AMPA seem to result from glyphosate and particularly GBF results rather than AMPA results. In fact, oxidative stress studies of AMPA are very limited. In the section on oxidative stress, IARC only cites one negative *in vitro* mammalian cell study of AMPA (Chaufan et al. 2014) and one positive *in vitro* mammalian cell study (Kwiatkowska et al. 2014). There is one other positive human cell study (Roustan et al. 2014) that was not cited; however, AMPA had unusually high toxicity in this report compared to other *in vitro* mammalian studies (see above) and no dose response was observed over an order of magnitude concentrations. The paucity and inconsistency of cited data does not seem to justify a conclusion of strong evidence for oxidative stress induction by AMPA.

Research on oxidative stress induced genotoxicity suggests that it is often a secondary response to toxicity and characterized by a threshold (Pratt & Barron 2003). Therefore the most appropriate conclusion supported by the oxidative stress data presented in IARC Monograph Section 4.2 is that there is not a strong WoE that glyphosate, GBFs, or AMPA produce oxidative damage to DNA that would lead to induction of endpoints predictive of a genotoxic hazard or act as a mechanism for the induction of cancer in experimental animals or humans.

Summary and conclusions

Detection of genotoxic activity or induction of oxidative stress/damage in any test conducted with a chemical does

not, *a priori*, mean that the agent has a carcinogenic potential, induces key events leading to tumor development or represents an *in vivo* genotoxic risk. A systematic and critical assessment of the WoE is required before genotoxic hazard and MoA conclusions can be reached. The IARC process leading to conclusions suggesting modes of action involving genotoxicity and oxidative stress was incomplete (excluding valuable data) and did not appear to critically evaluate some of the key studies it relied upon. A meaningful WoE evaluation depends on an assessment of all available data using an appropriate weighting process.

A number of reviews of the carcinogenicity, genotoxicity, and oxidative stress/damage for glyphosate, AMPA, and GBFs were available prior to the development of the IARC Glyphosate Monograph (see Introduction). These prior reviews included much of the data available to IARC reviewers involved in the evaluation presented in the IARC Monograph. In general, genetic toxicology data evaluated in these prior reviews all support a conclusion that glyphosate (and related materials) is inherently not genotoxic. The Expert Panel concluded that there is no new, valid evidence presented in the IARC Monograph that would provide a basis for altering these conclusions and that including the study results reviewed by Kier and Kirkland (2013) would provide considerable additional support to the conclusion of absence of inherent genotoxic potential.

- The Expert Panel concluded that glyphosate, GBFs, and AMPA genotoxicity response profiles are not consistent with characteristics of genotoxic carcinogens (Table 4).
- There is substantial evidence, particularly in bacterial reverse mutation assays, demonstrating that glyphosate, GBFs, or AMPA do not induce gene mutation from either direct or oxidative induced mechanisms.
- The evidence indicating that glyphosate can produce chromosomal aberrations in mammalian systems is very limited, conflicting, and potentially due to secondary mechanisms.
- The absence of evidence indicating that glyphosate or GBFs induced lesions characteristic of genotoxic carcinogens, in well-validated test systems with robust experimental protocols, invalidates conclusions that glyphosate or GBFs might act via a genotoxic MoA.
- The evidence for oxidative stress/damage as a mechanism or predictor of carcinogenesis is unconvincing. Repeated exposure to ROS most likely leads to adaptive responses, mitigating the mutagenicity of oxidative DNA lesions. Studies directed toward a better understanding of this relationship for glyphosate or GBF related exposures have not been reported.
- There is little or no reliable evidence that GBFs, at levels experienced across a broad range of end-user exposures, poses any human genotoxic hazard/risk.

The Expert Panel concluded that the IARC assessment of classifications regarding strong evidence of genotoxicity and oxidative stress capabilities of glyphosate, GBFs, and AMPA is not supported by the available data. A critical review of the complete dataset by the Expert Panel supports a conclusion that glyphosate (including GBFs and AMPA) does not pose a genotoxic hazard and therefore, should not be considered support for the classification of glyphosate as a genotoxic carcinogen. These conclusions are supportive of recent reviews that have occurred during the preparation of this review. A European Food Safety Authority peer review concluded that glyphosate is unlikely to pose a carcinogenic hazard to humans (EFSA 2015) and a Joint FAO/WHO Meeting on Pesticide Residues concluded that glyphosate is unlikely to be genotoxic at anticipated dietary exposures and unlikely to cause a carcinogenic risk to humans from dietary exposure (JMPR 2016).

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Declaration of interest

The employment affiliation of the authors is as shown on the cover page. However, it should be recognized that each individual participated in the review process and preparation of this paper as an independent professional and not as a representative of their employer. Gary Williams, David Brusick, and David Kirkland have previously served as independent consultants for the Monsanto Company on the European Glyphosate Task Force. Gary Williams has consulted for Monsanto on litigation matters involving glyphosate. Larry Kier was previously an employee of the Monsanto Company. Marilyn Aardema has not previously been employed in the Monsanto Company or previously been involved in any activity involving glyphosate and as such declares no potential conflicts of interest. Furthermore, other than Gary Williams, none of the aforementioned authors have been involved in any litigation procedures involving glyphosate.

The Expert Panel Members recruitment and evaluation of the data were organized and conducted by Intertek Scientific & Regulatory Consultancy (Intertek). The Expert Panelists acted as consultants for Intertek. Intertek (previously Cantox) is a consultancy firm that provides scientific and regulatory advice, as well as safety and efficacy evaluations for the chemical, food, and pharmaceutical industries. While Intertek Scientific & Regulatory Consultancy has not previously worked on glyphosate related matters for the Monsanto Company, previous employees of Cantox had worked in this capacity.

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Supplemental material

Supplemental material for this article is available online here.

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