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

OFFICE OF CHEMICAL SAFETY AND POLLUTION PREVENTION

MEMORANDUM

Date: December 12, 2017

SUBJECT: **Glyphosate** — Systematic Review of Open Literature

PC Code: 417300; 103601; 103603; 103604; 103605; 103607; 103608; 103613	**DP Barcode:** D417703
Decision No.: 487242	**Registration No.:** NA
Petition No.: NA	**Regulatory Action:** NA
Risk Assessment Type: NA	**Case No.:** NA
TXR No.: 0056885	**CAS No.:** 1071-83-6; 38641-94-0; 70393-85-0; 114370-14-8; 40465-76-7; 69254-40-6; 34494-04-7; 70901-12-1
MRID No.: NA	**40 CFR:** 180.364

FROM: Monique M. Perron, Sc.D., Toxicologist
Risk Assessment Branch I (RABI)
Health Effects Division (HED) (7509P)

THROUGH: Christine Olinger, Branch Chief
RABI (HED; 7509P)

TO: Tom Bloem, Chemist
Anwar Y. Dunbar, Ph.D., Pharmacologist
RABI (HED; 7509P)

Table of Contents

1. Introduction

The Agency strives to use high-quality studies when evaluating the hazard of pesticidal chemicals and considers a broad set of data during this process. A wide range of potential adverse effects are assessed using acute, subchronic, chronic, and route-specific studies predominately from studies with laboratory animals in addition to epidemiologic and human incident data. *In vitro* studies are typically considered as part of mode of action/adverse outcome pathway (MOA/AOP) analyses when *in vitro* to *in vivo* extrapolation (IVIVE) approaches are not sufficiently developed for deriving points of departure for regulatory risk assessment. All studies are thoroughly reviewed to ensure appropriate conduct and methodologies are utilized and that sufficient data and details are provided.

For all pesticides, there are toxicology data requirements that must be submitted to the Agency for registration. These studies, defined under the 40 CFR Part 158 Toxicology Data Requirements, provide information on a wide range of adverse health outcomes, routes of exposure, exposure durations, species, and lifestages. They typically follow Organization for Economic Cooperation and Development (OECD) accepted protocols and guidelines, which ease comparisons across studies and chemicals. Data may also be available to elucidate a chemical's hazard from the open scientific literature, structure activity relationships, physiologically-based pharmacokinetic (PBPK) or biological dose-response models, biomonitoring, or other exposure studies/analyses.

In 2012, OPP published a guidance document to provide guidance procedures for considering and using open literature toxicity studies to support human health risk assessment[1]. This guidance assists OPP scientists in their judgement of the scientific quality of open literature publications. More specifically, the document discusses how to screen open literature studies for journal articles/publications that are relevant to risk assessment, how to review potentially useful journal articles/publications and categorize them as to their usefulness in risk assessment, and how the studies may be used in the risk assessment.

In 2010, OPP developed a draft "Framework for Incorporating Human Epidemiologic & Incident Data in Health Risk Assessment" which provides the foundation for evaluating multiple lines of scientific evidence in the context of the understanding of the MOA/AOP (U.S. EPA, 2010). The draft framework, which includes two key components: problem formulation and use of the MOA/AOP frameworks, was reviewed favorably by the SAP in 2010 (FIFRA SAP, 2010). In 2016, a final version of the framework was published, which incorporated improvements based on recommendations from the SAP, public comments, and the experience gained since 2010 conducting assessments on several pesticides for which epidemiological data were available.

OPP's framework is consistent with updates to the World Health Organization/International Programme on Chemical Safety mode of action/human relevance framework, which highlight the importance of problem formulation and the need to integrate information at different levels of

[1] U.S. EPA (2012). *Guidance for considering and using open literature toxicity studies to support human health risk assessment.* http://www.epa.gov/pesticides/science/lit-studies.pdf

biological organization (Meek et al, 2014[2]). Consistent with recommendations by the NRC in its 2009 report on *Science and Decisions*[3], OPP's framework describes the importance of using problem formulation at the beginning of a complex scientific analysis. The problem formulation stage starts with planning dialogue with risk managers to identify goals for the analysis and possible risk management strategies. This initial dialogue provides the regulatory context for the scientific analysis and helps define the scope of such an analysis. The problem formulation stage also involves consideration of the available information regarding the pesticide use/usage, toxicological effects of concern and exposure pathways and duration along with key gaps in data or scientific information. Specific to glyphosate, the scoping document prepared for Registration Review (J. Langsdale; 3-JUN-2009; D362745) along with this memorandum represents the problem formulation analyses for the identification and evaluation of open literature studies with the potential to impact human health risk assessment.

In recent years, the National Academy of Sciences National Research Council (NRC) has encouraged the Agency to move towards systematic review processes to enhance the transparency of scientific literature reviews that support chemical-specific risk assessments to inform regulatory decision making[4]. The NRC defines systematic review as "a scientific investigation that focuses on a specific question and uses explicit, pre-specified scientific methods to identify, select, assess, and summarize the findings of similar but separate studies"[5]. Consistent with NRC's recommendations, EPA's Office of Chemical Safety and Pollution Prevention is currently developing policies and procedures in order to employ fit-for-purpose systematic reviews. As a result, more recent evaluations of open literature studies are starting to reflect this progression in the Agency's process. Similar to the framework for incorporating human epidemiologic and incident data, systematic review of the open literature begins with a problem formulation to determine the scope and purpose of the search. Studies are considered based on their relevance to answer specific questions and those studies deemed relevant are then further evaluated for their usefulness in risk assessment predominantly using much of the same criteria as those described in the 2012 open literature review guidance.

The purpose of this document is to detail two open literature searches conducted for glyphosate and the subsequent review of the studies gathered from these searches. The open literature was assessed for hazard identification and characterization purposes in order to identify studies that could potentially impact the human health risk assessment. For hazard identification purposes, *in vivo* studies were given more weight as generic IVIVE approaches are not developed for glyphosate and a PBPK model does not exist for glyphosate at this time. Studies concerning epidemiology, poisoning, or incidents were not considered as part of the reviews since a Tier II incident report was recently completed for glyphosate (S. Recore; 6-FEB-2014; D417808) that

[2] Meek ME, Boobis A, Cote I, Dellarco V, Fotakis G, Munn S, Seed J, Vickers C. 2014. New developments in the evolution and application of the WHO/IPCS framework on mode of action/species concordance analysis. J Appl Toxicol. 2014 Jan;34(1):1-18.
[3] NRC (National Research Council). 2009. Science and decisions: Advancing risk assessment. Washington, DC: The National Academies Press. http://www.nap.edu/openbook.php?record_id=12209

[4] NRC 2011. "Review of the Environmental Protection Agency's Draft IRIS Assessment of Formaldehyde"; NRC 2014. "Review of EPA's Integrated Risk Information System (IRIS) Process"
[5] NRC (2014). Review of EPA's Integrated Risk Information System (IRIS) process. Washington, DC: The National Academies Press. http://www.nap.edu/catalog.php?record_id=18764

evaluated available human epidemiologic and incident data. Furthermore, epidemiology studies were also considered as part of the Agency's evaluation of the human carcinogenic potential of glyphosate (D444689, 12-DEC-2017; TXR# 0057688 and J. Rowland; 1-OCT-2015; TXR #0057299).

2. Document Overview

As part of the draft risk assessment for Registration Review, the Agency collaborated with Health Canada's Pest Management Regulatory Agency (PMRA) in 2012 to conduct a literature search and review for glyphosate using the 2012 open literature review guidance. A subsequent search of the open literature was conducted more recently by the Agency to supplement the 2012 joint review with PMRA. This more recent search reflects the Agency's movement toward systematic review and OPP's efforts to standardize literature review documentation. Due to the large number of studies reviewed as part of the 2012 joint review with PMRA, the format of that review was not updated to reflect the more current approaches utilized in the more recent search; therefore, results of these searches will be described and presented differently in this document. The primary goal for both of these searches was to identify relevant and appropriate open literature studies that had the potential to impact human health risk assessment. This document presents the results of these open literature searches and is organized according to the following:

- Section 3 provides information on how each search was conducted and additional details related to the review of potentially relevant studies;
- Section 4 presents the results of the 2012 and 2015 searches;
- Section 5 provides information found regarding whole animal exposures to commercial formulations given the large number of studies that were seen in the open literature that used commercial formulations;
- Section 6 discusses the impact of the literature searches on the human health risk assessment for glyphosate;
- Appendix A is a table with the summary of findings for the joint review with PMRA;
- Appendix B is a list of references included in the joint review with PMRA;
- Appendix C is a compilation of the data evaluation records (DERs) generated for the joint review with PMRA;
- Appendix D is a table with the literature studies obtained from the recent search of PubMed and screening comments.

3. Search Methods

3.1 Open literature search with PMRA

The search was conducted for abstracts, articles, and articles in press, books and reviews at http://www.scirus.com under the following subject areas: Life Sciences, Agriculture and Biological Sciences, Pharmacology, Medicine, and Neuroscience. Search results that did not contain the keywords on their abstracts were not reported and duplicates were removed. The

same keywords were then searched on PubMed. The following is a list of the search terms used to address various durations and endpoints of toxicity:

1. Reproduction/Developmental Toxicity (Date of Search: Jan-Feb, 2012)
- In search bar, the keywords Glyphosate and Reproduct* toxicity were entered.
- Other search terms used were Glyphosate and developmental toxicity.

2. Short-term Toxicity/Long term Toxicity/Oncogenicity (Date of Search: December, 2011-Feb, 2012)
- In search bar, the keywords Glyphosate and chronic toxicity studies were entered.
- The search results captured in the previous searches (e.g. Glyphosate and Reproduct* toxicity) were not reported.
- Searching the keywords Glyphosate and Tumours or Tumors did not result in meaningful results. Results were not considered relevant to human health (e.g. Impact of glyphosate on the development, fertility and demography of Chrysoperla externa (Neuroptera: Chrysopidae): ecological approach)
- Other terms searched were glyphosate and mammalian toxicity, glyphosate and short term toxicity studies, and glyphosate and toxicity.
- Regulatory reports that were not captured in scirus.com were also included in this section because they contain several published toxicology studies.

3. In Vitro/Genotoxicity/Endocrine Studies (Date of Search: Nov-Dec, 2011)
- In search bar, the keywords Glyphosate and Endocrine were entered.
- The search results captured in the previous searches (e.g. Glyphosate and Reproduct* toxicity) were not reported.
- Other terms searched were Glyphosate and genotoxicity.

4. Epidemiology Studies/Incident Reports
- In search bar, the keywords Glyphosate and Epidemiology were entered.
- The search results captured in the previous searches (e.g. Glyphosate and Reproduct* toxicity) were not reported.

An initial screening process was undertaken to identify the published articles that are most relevant and appropriate for the purposes of addressing the critical aspects of hazard identification and characterization for human health risk assessment. Criteria from the OPP 2012 open literature review guidance were then used to identify suitable studies for hazard assessment. Eligible journal articles/publications are reviewed to ensure general information has been included that is considered important in determining the reliability and utility of a study in human health risk assessment. This information covers a range of factors that are commonly evaluated as part of guideline study reviews, such as (but not limited to) the nature of the test substance, test organism details (*e.g.*, species, age, sex, lifestage, etc.), adequate sample size, adequate dose levels, appropriate husbandry conditions, exposure details (*i.e.*, method, route, frequency, and length of treatment period), and suitable controls.

PMRA generated DERs for each eligible study listing study limitation/deficiencies and categorized studies as acceptable or invalid (Appendix C). Acceptable studies were then further

categorized as appropriate for quantitative or qualitative use in risk assessment. Studies found to be acceptable and appropriate for quantitative use were evaluated to determine no observed adverse effect levels (NOAELs) and lowest observed adverse effect levels (LOAELs).

The Agency conducted secondary reviews of the DERs primarily focusing on whether the study would be considered acceptable or unacceptable (considered equivalent to the PMRA categories of acceptable or invalid for this review). For studies found to be acceptable and appropriate for quantitative, NOAEL and LOAEL values were evaluated based on current Agency policies and approaches in hazard characterization. If the Agency disagreed with the values set by PMRA, then separate NOAEL and LOAEL values were reported for each agency. Studies concerning epidemiology, poisoning, or incidents were also removed at this time.

3.2 Recent Systematic Review

To obtain literature studies that have been published since the collaborative search with PMRA, a search was conducted on October 5, 2015 for studies published from January 1, 2012 to October 5, 2015 using the following search string in PubMed:

(((((glyphosate) NOT plants)) NOT frogs) NOT tadpoles AND (("2012/01/01"[PDat] : "2015/10/05"[PDat]))

The list was then cross-referenced with other literature studies submitted during that time to the Agency by non-profit groups or members of the public. Titles and abstracts were initially screened to identify journal articles that were not within the scope of the search due to the subject of the research (*e.g.*, ecological and fate studies, monitoring data, crop composition studies, pest management studies) or the type of article (*e.g.*, review, commentary, editorial, article retraction). Full articles were further screened if necessary to determine their relevance to human health risk assessment and to determine whether the journal article would be considered appropriate for use in risk assessment. Full articles that were not publically available or were not available in English were not considered. Studies concerning epidemiology, poisoning, or incidents were not considered.

4. Results

4.1 Open literature search with PMRA

PMRA provided DERs for 67 studies (Appendix C) obtained from 62 individual references (Appendix B). Of these, 17 studies related to poisoning/incidents, biomonitoring data, and epidemiological studies were not included in this review[6] since they were evaluated as part of the Tier II incident report (S. Recore; 6-FEB-2014; D417808). The U.S. EPA conducted secondary reviews and generally agreed with PMRA's conclusions regarding the validity and potential use of the remaining studies. A summary of the findings can be found in Table A-1 of Appendix A, which lists the PMRA and EPA categorizations for each study and some of the noted limitations/deficiencies if the study was found to be invalid or unacceptable.

[6] References in Appendix B not included: 1, 5, 11, 13, 15, 20, 31, 32, 35, 38, 40, 51, 54, 55, 57, 58, and 62.

The majority of these literature studies were found to be unacceptable for use in the U.S. EPA's glyphosate Registration Review draft human health risk assessment for a variety of reasons (see Table A-1 of Appendix A and Appendix C). For example, 6 studies did not meet the minimum criteria to be considered eligible (*e.g.*, the study was not found to be the primary source of the data, was not publicly available, or not presented as a full article). Of the studies eligible for further review, the most common limitations/deficiencies were related to the nature of the test substance(s) used for exposure. Most studies used commercial formulations or dilutions; however, direct measurements of the active ingredient were not conducted in order to determine actual dose concentrations and/or identification information was not provided for the formulation used (e.g., EPA registration number). As a result, potential effects could not be attributed to defined glyphosate exposure concentrations. Additionally, limited dosing, small sample sizes, and lack of test chemical purity was often noted in several of the studies.

Only a limited number of the reviewed literature studies for glyphosate were deemed acceptable and appropriate for qualitative or quantitative risk assessment purposes. The only studies found to be appropriate for quantitative use were the 90-day oral toxicity studies in rats and mice from the NTP Technical Report (Reference #43 in Appendix B). In these studies, cytoplasmic alterations of the salivary gland were observed in both species. The Agency set NOAELs for this effect at ~400 and >1000 in rats and mice, respectively. Alterations at lower doses were scored as minimal and not considered adverse. These dose levels are well above the point of departures currently used for risk assessment. Therefore, none of the studies in this joint PMRA 2012 review will have an impact on the hazard characterization or draft human health risk assessment for glyphosate.

4.2 Recent systematic review

The literature search yielded 392 articles. This list was then cross-referenced with other studies submitted during that time to the Agency by non-profit groups or members of the public and another 7 studies were added for review bringing the total number of articles to 399 (Appendix D). Since the goal of the literature search was to identify relevant and appropriate open literature studies that had the potential to impact human health risk assessment, most of the studies (288 articles) were not considered to be within the scope of the search due to the subject of the research (*i.e.*, ecological and fate studies, crop composition studies, pest management studies, method generating, hypothesis generating, exposure and monitoring) or not relevant in general. Additionally, 26 articles were not appropriate due to the type of article (*i.e.*., review, commentary, editorial, article retraction, news article, abstract only, not available in English). Another 27 articles were not considered because they concerned epidemiology, poisoning, and/or incidents.

The remaining studies were further screened for relevance to human health and similar to the search conducted with PMRA, almost all of the potentially relevant studies used commercial formulations; however, direct measurements of the active ingredient were not conducted in order to determine actual dose concentrations and/or identification information was not provided for the formulation used. As a result, potential effects could not be attributed to defined glyphosate

exposure concentrations. None of the studies from this more recent review were found to have an impact on the hazard characterization or draft human health risk assessment for glyphosate.

5. *In vivo* Studies with Commercial Formulations

The Agency recognizes that a multitude of studies have been performed using commercial formulations containing glyphosate. The majority of these are *in vitro* studies, which are difficult to translate into *in vivo* effects where metabolism and clearance would play a large role in potential toxicity. As mentioned previously, i*n vitro* studies are typically considered as part of MOA/AOP analyses when *in vitro* to *in vivo* extrapolation (IVIVE) approaches are not sufficiently developed for deriving points of departure for regulatory risk assessment. Consequently, *in vivo* studies are given more weight as generic IVIVE approaches are still under development for glyphosate and a PBPK model does not exist for glyphosate at this time. Only a limited number of *in vivo* studies (13 studies) were identified as part of both the 2012 joint review with PMRA and the more recent 2015 systematic review (Table 1). Studies conducted via a route that was not relevant for human exposure were not included (i.e., intraperitoneal injection).

The 13 *in vivo* studies were evaluated using similar criteria outlined in the 2012 open literature review guidance for evaluating the acceptability of open literature studies. The Agency strives to use high-quality studies that follow acceptable conduct and contain all the sufficient information needed to evaluate potential adverse effects from exposure to a pesticidal active ingredient. This is also the case for a study conducted using a commercial formulation. Consistent with guidance to determine whether a study meets the criteria outlined in guidelines for pesticide testing, there is general information that is considered important when determining the reliability and utility of an open literature study. A study could be considered of questionable reliability and utility based on a single factor or, more typically, several issues may be combined to lead to this conclusion. As such, none of the 13 studies were found to be of adequate quality in order to be further reviewed for use in risk assessment. Overall, the following were common limitations/deficiencies seen in these studies:

- The majority of the studies reported using a commercial formulation and often referred to the general product name (e.g., Roundup); however, there are numerous formulations with glyphosate as the active ingredient and reporting identification information (including the full product name and EPA registration number) is important since this information allows the Agency to ascertain the exact formulation used in a study and determine all of its chemical components.
- Exposure conditions were not adequately described or documented, especially in the case of gavage studies.
- Data was only presented as graphs and often measures of variability were not included.
- Sample sizes were considered too small for the type of study conducted and/or not reported for all lifestages (e.g., fetuses).
- Only one dose was investigated to look at an observed effect, which does not allow for dose-response evaluation of the effect.
- Age and overall health of animals prior to commencing a study was not reported.

Most of these studies with commercial formulations focused on clinical chemistry measurements (i.e., enzymes, hormones, electolytes) or histopathological examinations (without reporting severity) making it difficult to determine the adversity of the results. The relationship between any changes noted in these effects and possible adverse apical outcomes from commercial formulations has not been established. As described in the NRC report, "Toxicity Testing in the 21st Century"[7], to develop a MOA/AOP not only is it necessary to establish plausible relationships among the key events, but quantitative relationships also need to be established. In other words, how much of a change in one key event is needed to result in an adverse effect at the next level of biological organization? Thus, certain exposures to a chemical may impact normal physiological responses in a way that may not necessarily be adverse, and thus, the MOA/AOP concept requires an understanding of adaptive/homeostatic capacity of biological systems and their limits, relative to concentration and duration of exposure. Without an MOA/AOP understanding or even a potentially solid hypothesis, pertubations in physiology cannot be interpreted for risk assessment without understanding how these changes lead to adverse outcomes.

[7] National Research Council (NRC). 2007. Toxicity Testing in the 21st Century: A Vision and a Strategy. Washington, D.C. The National Academies Press.

Table 1. Review of *in vivo* journal articles performed with commercial formulations containing glyphosate.

Journal Article	Benedetti et al. 2004	Beuret et al. 2005	Caglar and Kolankaya 2008	Cattani et al. 2014	Dallegrave et al. 2003	Dallegrave et al. 2007
Test Substance						
Source of test substance reported?	Yes (Biocarb)	Yes (Herbicygon)	Yes (Roundup)	Yes (Roundup Original)	Yes (Roundup)	Yes
Adequate information to identify specific formulation (full product name, registration number, etc.)?	No	No	No	No	No	No
Analysis of glyphosate or other components?	No	No	No	No	No	No
Vehicle (if applicable) reported?	Yes	Yes	Yes	Yes	Yes	Yes
Test Species						
Species, age, sex, size, health, and lifestage reported?	No	No	No	No	No	No
If not, what information is missing?	Health	Age, health	Health, species identified as Wistar and Sprague-Dawley	Age, health, size	Health	Health
Was only one sex tested?	Yes, males	Yes, females	No	Yes, females	Yes, females	Yes, females
Was the number of test animals reported?	Yes	Yes	No	Yes	Yes	Yes
Is the number of animals used sufficient?	Yes	Yes (lower than typical developmental, but only looked at enzymes)	Unknown	No (only 4 dams)	Yes (but slightly lower than typical developmental)	Yes (but slightly lower than typical developmental)
Animal Husbandry						
Were adequate husbandry conditions presented?	No	No	No	No	No	No
If not, what information is missing?	Source of feed, diet description	Humidity, nature and composition of bedding, container dimensions	Source of feed, diet description	Humidity, nature and composition of bedding, container dimensions,	Humidity, source of feed, diet description	Humidity, diet description

Journal Article	Benedetti et al. 2004	Beuret et al. 2005	Caglar and Kolankaya 2008	Cattani et al. 2014	Dallegrave et al. 2003	Dallegrave et al. 2007
Test Substance						
				diet description, number per cage		
Exposure Conditions						
Were the exposure conditions adequately described?	Yes	Yes	No (assumed to be by drinking water)	Yes	Yes	Yes
Was a control used?	Yes	Yes	Yes	Yes	Yes	Yes
Was the control adequate?	Yes	Yes	Yes	Yes	Yes	Yes
Was there more than one dose tested to evaluate the dose response nature of the effects?	Yes	No	Yes	No	Yes	Yes
Were the animals assigned to treatment groups randomly?	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown
Toxicity Effects (Non-histopathological)						
Were the toxicological effects reported adequately described (i.e., nature, incidence, time, severity, and duration)?	Yes	Yes	Yes	Yes	Yes	Yes
If not, what information is missing or incomplete?	-	-	-	-	-	-
Were the research assessors blinded to the treatment?	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown
Toxicity Effects (Histopathological)						
Were histopathological evaluations performed?	Yes	No	Yes	No	No	Yes
Were the tissues properly fixed?	Yes	-	Yes	-	-	Yes
Were the histopathological findings properly described, including the severity and incidence?	No	-	No	-	-	No
Were the research assessors blinded to the treatment?	Unknown	-	Unknown	-	-	Unknown
Statistics						

Journal Article	Benedetti et al. 2004	Beuret et al. 2005	Caglar and Kolankaya 2008	Cattani et al. 2014	Dallegrave et al. 2003	Dallegrave et al. 2007
Test Substance						
Are the statistical methods properly described?	Yes	Yes	Yes	Yes	Yes	Yes
Does the data include a measure of the variability (SD or SE)?	Yes	Yes	Yes	Yes	Yes	Yes
Study Results/Discussion						
Do the authors provide a clear summary of the data?	Yes	Yes	Yes	Yes	Yes	Yes
Does the report include clear conclusions drawn from the analysis?	Yes	Yes	Yes	Yes	Yes	Yes
Overall, do you feel like the study is of adequate quality to be reviewed for use in risk assessment?	No	No	No	No	No	No
Comments	Utility of results questionable given relatively small changes in liver enzymes and presence of Kupffer cells only at highest dose (487 mg/kg), full identification of formulation needed	Only one dose tested; no description of formulation used; adversity of results questionable without relating to downstream effects	Exposure conditions were not clear; number of test animals not reported, inconsistencies noted (e.g., rats listed as 2 different species); relatively no change in clinical chemistry measurements; severity not reported	Only one dose tested; utility of results unclear; data only presented as graphs	Doses and observed effects were above those relevant for risk assessment (≥500 mg/kg), full identification of formulation needed; some data only presented as graphs	Severity of histopathological findings not reported, relatively small changes and/or no dose response with treatment for many effects evaluated, full identification of formulation needed

Table 1 Cont'd.							
Journal Article	Daruich et al. 2001	Jasper et al. 2012	Romano et al. 2010	Romano et al. 2012	Seralini et al. 2014	Tizhe et al. 2014a	Tizhe et al. 2014b
Test Substance							
Source of test substance reported?	Yes (Herbicygon)	Yes (Roundup Original)	Yes (Roundup Transorb)	Yes (Roundup Transorb)	Yes (WeatherMAX)	Yes (Bushfire®)	Yes (Bushfire®)
Adequate information to identify specific formulation (full product name, registration number, etc.)?	No	No	No	No	Yes (WeatherMAX; 540 g/L glyphosate, EPA Reg. 524-537)	No	No
Analysis of glyphosate or other components?	No	No	No	No	No	No	No
Vehicle (if applicable) reported?	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Test Species							
Species, age, sex, size, health, and lifestage reported?	No	No	Yes	No	No	No	No
If not, what information is missing?	Age, health	Health	Size, health	Body weight, Health, Lifestage (not reported for dams)	Size, health	Age, health, lifestage	Age, health, lifestage
Was only one sex tested?	Yes, females	No	Yes, males	Yes, females	No	No	No
Was the number of test animals reported?	Yes (for dams); Fetal number unknown	Yes	Yes	Yes	Yes	Yes	Yes
Is the number of animals used sufficient?	Yes for dams (lower than typical developmental, but only looked at enzymes); unknown for fetuses	Yes	Yes	No (only 12 dams)	No (only 10/sex/group)	Yes	Yes
Animal Husbandry							
Were adequate husbandry conditions presented?	No	No	No	No	Yes	No	No

Table 1 Cont'd.							
Journal Article	Daruich et al. 2001	Jasper et al. 2012	Romano et al. 2010	Romano et al. 2012	Seralini et al. 2014	Tizhe et al. 2014a	Tizhe et al. 2014b
If not, what information is missing?	Humidity, nature and composition of bedding, container dimensions	Container dimensions, number per cage, source of animal feed	Humidity, nature and composition of bedding, container dimensions, number per cage, source of animal feed	Humidity, nature and composition of bedding, container dimensions, number per cage, source of animal feed	-	Ambient temperature and humidity, photoperiod, nature and composition of bedding, container dimensions, number per cage, source of animal feed	Ambient temperature and humidity, photoperiod, nature and composition of bedding, container dimensions, number per cage, source of animal feed
Exposure Conditions							
Were the exposure conditions adequately described?	Yes	No	Yes	Yes	Yes	No	No
Was a control used?	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Was the control adequate?	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Was there more than one dose tested to evaluate the dose response nature of the effects?	Yes	Yes	Yes	No	Yes	Yes	Yes
Were the animals assigned to treatment groups randomly?	Unknown	Unknown	Yes	Unknown	Yes	Yes	Yes
Toxicity Effects (Non-histopathological)							
Were the toxicological effects reported adequately described (i.e., nature, incidence, time, severity, and duration)?	Yes	Yes	Yes	Yes	No	No	NA
If not, what information is missing or incomplete?	-	-	-	-	Not all data shown, limited description	Data values not provided	-
Were the research assessors blinded to the treatment?	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown
Toxicity Effects (Histopathological)							
Were histopathological evaluations performed?	No	Yes	Yes	Yes	Yes	No	Yes
Were the tissues properly fixed?	-	Yes	Yes	Yes	Yes	-	Yes
Were the histopathological findings properly described,	-	Yes	Yes	Yes	Yes	-	No

Table 1 Cont'd.							
Journal Article	Daruich et al. 2001	Jasper et al. 2012	Romano et al. 2010	Romano et al. 2012	Seralini et al. 2014	Tizhe et al. 2014a	Tizhe et al. 2014b
including the severity and incidence?							
Were the research assessors blinded to the treatment?	-	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown
Statistics							
Are the statistical methods properly described?	Yes	Yes	Yes	Yes	Yes	Yes	No statistics performed – Only histopathology
Does the data include a measure of the variability (SD or SE)?	Yes	Yes	Yes	Yes	Yes (for some measurements)	Yes	-
Study Results/Discussion							
Do the authors provide a clear summary of the data?	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Does the report include clear conclusions drawn from the analysis?	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Overall, do you feel like the study is of adequate quality to be reviewed for use in risk assessment?	No	No	No	No	No	No	No
Comments	Full identification of formulation needed, number of fetal animals unknown, small changes and/or no dose response with treatment, adversity of enzyme changes alone difficult to determine without downstream measurements, some data only presented as graphs	Full identification of formulation needed, no information provided on gavage volume	Full identification of formulation needed, discrepancies in sample size between experimental description and results, no information about which pups came from which litters.	Full identification of formulation needed, only 12 dams were used, not all data shown (e.g., body weights)	Not all data shown, some data presented only as graphs or percentages, measures of variability only on some measurements, small sample size for a long term exposure cancer study	Full identification of formulation needed, only graphs and percentages were provided for clinical chemistry data, exposure conditions were unclear (i.e., exposure volumes for gavage), relatively small changes in formulation treatment groups.	Full identification of formulation needed, severity/incidence of histopathology not provided, exposure conditions unclear (i.e., exposure volumes for gavage).

6. Impact on Glyphosate Human Health Risk Assessment

Over 450 open literature journal articles were considered as part of this review. Only a limited number of these studies were deemed acceptable and appropriate for consideration in risk assessment. The only studies found to be appropriate for quantitative use identified NOAELs at doses well above the point of departures currently used for risk assessment. As a result, there was no impact on the hazard characterization or draft human health risk assessment for glyphosate. The Agency will continue to monitor the open literature for studies that use scientifically sound and appropriate methodology and relevant routes of exposure that have the potential to impact the risk evaluation of glyphosate.

Appendices:

Appendix A: Summary of the findings for the joint review with PMRA

Appendix B: Studies included in the joint review with PMRA

Appendix C: Data Evaluation Records (DERs) generated for the joint review with PMRA

Appendix D: Literatures studies obtained from searching PubMed for recent systematic review

Appendix A: Summary of the findings for the joint review with PMRA

Table A-1. Glyphosate Literature Search with PMRA — Primary and Secondary Review Findings				
Study Title (Reference # in Appendix B)	**Study Type**	**Primary Review (PMRA)**	**Secondary Review (U.S. EPA)**	**Limitations/Deficiencies[2]**
NTP Technical Report on Toxicity Studies of Glyphosate Administered in Dosed Feed to F344/N Rats and B6C3F1 Mice. (Ref. No. 43)	90-day oral (rat)	Acceptable even though not conducted according to guideline requirements; appropriate for quantitative use	Acceptable; appropriate for quantitative use	Not Applicable
NTP Technical Report on Toxicity Studies of Glyphosate Administered in Dosed Feed to F344/N Rats and B6C3F1 Mice. (Ref. No. 43)	90-day oral (mice)	Acceptable even though not conducted according to guideline requirements; appropriate for quantitative use	Acceptable; appropriate for quantitative use	Not Applicable
NTP Technical Report on Toxicity Studies of Glyphosate Administered in Dosed Feed to F344/N Rats and B6C3F1 Mice. (Ref. No. 43)	Mechanistic (rat)	Acceptable; appropriate for qualitative use	Acceptable; appropriate for qualitative use	Not Applicable
An Evaluation of the Genotoxic Potential of Glyphosate. (Ref. No. 34)	Bacterial reverse mutation assay	Acceptable; satisfies guideline requirements	Acceptable	Not Applicable
An Evaluation of the Genotoxic Potential of Glyphosate. (Ref. No. 34)	In vitro mammalian cell assay	Acceptable; satisfies guideline requirements however some deficiencies present	Acceptable	Not Applicable
An Evaluation of the Genotoxic Potential of Glyphosate. (Ref. No. 34)	In vivo cytogenetics	Acceptable; satisfies guideline requirements	Acceptable	Not Applicable
Toxicokinetics of Glyphosate and Its Metabolite Aminomethyl Phosphonic Acid in Rats (Anadon et al. 2009). (Ref. No. 3)	Metabolism	Acceptable even though not conducted according to guideline requirements; appropriate for qualitative use	Acceptable; appropriate for qualitative use	Not Applicable
NTP Technical Report on Toxicity Studies of Glyphosate Administered in Dosed Feed to F344/N Rats and B6C3F1 Mice. (Ref. No. 43)	Metabolism	Acceptable even though not conducted according to guideline requirements; appropriate for qualitative use	Acceptable; appropriate for qualitative use	Not Applicable
Glyphosate Impairs Male Offspring Reproductive Development by Disrupting Gonadotropin Expression. (Ref. No. 53)	NA	Invalid (inappropriate for quantitative or qualitative use in risk assessment because it is of	Unacceptable (inappropriate for quantitative or qualitative use in risk assessment because it is of	- The test substance was a glyphosate based commercial formulation.

Table A-1. Glyphosate Literature Search with PMRA — Primary and Secondary Review Findings				
Study Title (Reference # in Appendix B)	**Study Type**	**Primary Review (PMRA)**	**Secondary Review (U.S. EPA)**	**Limitations/Deficiencies[2]**
		insufficient quality and lacks scientific defensibility)	insufficient quality and lacks scientific defensibility)	- The percent active ingredient was not indicated/measured and other components were not identified. - Only one dose group was used.
Vitamin C and E reverse effect of herbicide-induced toxicity on human epidermal cells HaCaT: a biochemometric approach. (Ref. No. 25)	NA	Invalid	Unacceptable (inappropriate for quantitative or qualitative use in risk assessment because it is of insufficient quality and lacks scientific defensibility)	- The percent active ingredient was not indicated/measured and other components were not identified. - Inadequate description of the data in the results section as the IC_{50} values could not be obtained for glyphosate alone (the active ingredient) before the addition of Vitamin C or E. - It is in vitro effects with in vivo toxicity.
Glyphosate-based pesticides affect cell cycle regulation. (Ref. No. 37)	NA	Invalid	Unacceptable (inappropriate for quantitative or qualitative use in risk assessment because it is of insufficient quality and lacks scientific defensibility)	- The test substance was a glyphosate based commercial formulation. - The percent active ingredient was not indicated/measured and other components were not identified. - It is difficult to extrapolate in vitro effects with in vivo toxicity.
A glyphosate-based herbicide induces necrosis and apoptosis in mature rat testicular cells in vitro and testosterone decrease at lower levels. (Ref. No. 16)	NA	Invalid or of little utility for hazard assessment	Unacceptable (inappropriate for quantitative or qualitative use in risk assessment because it is of insufficient quality and lacks scientific defensibility)	- The percent active ingredient was not indicated/measured and other components were not identified. - It is difficult to extrapolate in vitro effects with in vivo toxicity. - The study report did not discuss whether the tested concentrations of glyphosate would produce cytotoxicity and impact the results of this study
Placental passage of benzoic acid, caffeine and glyphosate in an ex vivo human perfusion system. (Ref. No. 42)	NA	Invalid or of little utility for hazard assessment	Unacceptable (inappropriate for quantitative or qualitative use in risk assessment because it is of insufficient quality and lacks scientific defensibility)	- The purity of glyphosate was not provided. - It is difficult to extrapolate in vitro effects with in vivo toxicity.
Alteration of estrogen-regulated gene expression in human cells induced by agricultural and horticultural herbicide glyphosate. (Ref. No. 29)	NA	Invalid or of little utility for hazard assessment	Unacceptable (inappropriate for quantitative or qualitative use in risk assessment because it is of insufficient quality and lacks scientific defensibility)	- The purity of glyphosate was not provided. - The cell lines used were not adequately characterized. - It is difficult to extrapolate in vitro effects with in vivo toxicity. - The retail product used as stock was not identified and the concentration of glyphosate was not measured.

Table A-1. Glyphosate Literature Search with PMRA — Primary and Secondary Review Findings				
Study Title (Reference # in Appendix B)	**Study Type**	**Primary Review (PMRA)**	**Secondary Review (U.S. EPA)**	**Limitations/Deficiencies[2]**
The oral and intra-tracheal toxicities of Roundup and its components to rats. (Ref. No. 2)	NA	Invalid (inappropriate for quantitative or qualitative use in risk assessment because it is of insufficient quality and lacks scientific defensibility); provides useful info about effects of surfactant	Unacceptable (inappropriate for quantitative or qualitative use in risk assessment because it is of insufficient quality and lacks scientific defensibility)	- The study included in addition to glyphosate, a surfactant, and a glyphosate commercial formulation without measurements of glyphosate. - The purity of the test substances were not specified. - The study did not evaluate multiple dose levels (could not detect a dose response, and therefore, it is not possible to identify a NOAEL/LOAEL). - Only 8 animals/group were used, which reduces the strength of statistical analysis. - At time of assignment, the range of body weights was larger than expected (difference of 100g). - Study did not include body weight, body weight gain and food consumption data.
Glyphosate formulations induce apoptosis and necrosis in human umbilical, embryonic and placental cells. (Ref. No. 7)	NA	Invalid (inappropriate for quantitative or qualitative use in risk assessment because it is of insufficient quality and lacks scientific defensibility)	Unacceptable (inappropriate for quantitative or qualitative use in risk assessment because it is of insufficient quality and lacks scientific defensibility)	- The study included glyphosate formulations, in addition to glyphosate itself, surfactant and primary metabolite, AMPA. - The purities of test substances were not specified. - Data was shown only in a graph format, without access to raw data tables. - It is difficult to extrapolate in vitro effects with in vivo toxicity.
Roundup revelation: weed killer adjuvants may boost toxicity. (Ref. No. 12)	NA	Invalid (inappropriate for quantitative or qualitative use in risk assessment because it is of insufficient quality and lacks scientific defensibility)	Unacceptable (inappropriate for quantitative or qualitative use in risk assessment because it is of insufficient quality and lacks scientific defensibility)	- The reference is for a commentary on a study in Environmental Health Perspectives (see Differential Effects of Glyphosate and Roundup on Human Placental Cells and Aromatase). - The reference has incomplete data.
Pre- and post-natal toxicity of the commercial glyphosate formulation in Wistar rats. (Ref. No. 18)	NA	Invalid (inappropriate for quantitative or qualitative use in risk assessment because it is of insufficient quality and lacks scientific defensibility)	Unacceptable (inappropriate for quantitative or qualitative use in risk assessment because it is of insufficient quality and lacks scientific defensibility)	- The test substance was a glyphosate based commercial formulation. - The percent active ingredient was not indicated/measured or measured and other components were not identified. - Only 5 animals per dose were observed at microscopic level.
Morphological damages of a glyphosate-treated keratinocyte cell line revealed by a micro- to nanoscale microscopic investigation. (Ref. No. 21)	NA	Invalid (inappropriate for quantitative or qualitative use in risk assessment because it is of insufficient quality and lacks scientific defensibility)	Unacceptable (inappropriate for quantitative or qualitative use in risk assessment because it is of insufficient quality and lacks scientific defensibility)	- Method has not been evaluated for reproducibility and validity. Graded scale of effects was not indicated. - It is difficult to extrapolate in vitro effects with in vivo toxicity and to determine a NOAEL value.

Table A-1. Glyphosate Literature Search with PMRA — Primary and Secondary Review Findings				
Study Title (Reference # in Appendix B)	**Study Type**	**Primary Review (PMRA)**	**Secondary Review (U.S. EPA)**	**Limitations/Deficiencies[2]**
				- Purity of test substance was not specified.
Glyphosate-based herbicides are toxic and endocrine disruptors in human cell lines. (Ref. No. 23)	NA	Invalid (inappropriate for quantitative or qualitative use in risk assessment because it is of insufficient quality and lacks scientific defensibility)	Unacceptable (inappropriate for quantitative or qualitative use in risk assessment because it is of insufficient quality and lacks scientific defensibility)	- The test substances were glyphosate based on commercial formulations and glyphosate as negative control. - The percentages of active ingredient were indicated but purity was not specified. Other formulation components of the formulation were not identified.
Dig 1 protects against cell death provoked by glyphosate-based herbicides in human liver cell lines. (Ref. No. 24)	NA	Invalid (inappropriate for quantitative or qualitative use in risk assessment because it is of insufficient quality and lacks scientific defensibility)	Unacceptable (inappropriate for quantitative or qualitative use in risk assessment because it is of insufficient quality and lacks scientific defensibility)	- The test substances were glyphosate based commercial formulations and a new drug Dig 1. - The percentages of active ingredient were indicated, but purity of glyphosate was not specified. Other formulation components were not identified. - The study focused more on the protective effect of Dig 1, than toxicity of glyphosate.
Mechanism of toxicity of commercial glyphosate formulations: How important is the surfactant? (Ref. No. 27)	NA	Invalid (inappropriate for quantitative or qualitative use in risk assessment because it is of insufficient quality and lacks scientific defensibility)	Unacceptable (inappropriate for quantitative or qualitative use in risk assessment because it is of insufficient quality and lacks scientific defensibility)	- Only an abstract is available. - The test substance is poorly characterized.
Prepubertal exposure to commercial formulation of the herbicide glyphosate alters testosterone levels and testicular morphology. (Ref. No. 52)	NA	Invalid (inappropriate for quantitative or qualitative use in risk assessment)	Unacceptable (inappropriate for quantitative or qualitative use in risk assessment because it is of insufficient quality and lacks scientific defensibility)	- The test substance was a glyphosate-based commercial formulation - No information was provided on the inert ingredients. - Histology was restricted to an examination of the testes and adrenal glands.

Table A-1. Glyphosate Literature Search with PMRA — Primary and Secondary Review Findings				
Study Title (Reference # in Appendix B)	**Study Type**	**Primary Review (PMRA)**	**Secondary Review (U.S. EPA)**	**Limitations/Deficiencies[2]**
Effect of the herbicide glyphosate on liver lipoperoxidation in pregnant rats and their fetuses. (Ref. No. 9)	NA	Invalid (inappropriate for quantitative or qualitative use in risk assessment)	Unacceptable (inappropriate for quantitative or qualitative use in risk assessment because it is of insufficient quality and lacks scientific defensibility)	- The test substance was a glyphosate-based commercial formulation. - The percent active ingredient and purity were not stated/measured. - Histopathology was not performed on the maternal and fetal livers that were collected. No other organs were examined. - A single treated group, rather than a range of dosage groups, was compared to the control. - No rationale is provided for dose selection.
A generational study of glyphosate-tolerant soybeans on mouse fetal, postnatal, pubertal and adult testicular development. (Ref. No. 10)	NA	Invalid (inappropriate for quantitative or qualitative use in risk assessment)	Unacceptable (inappropriate for quantitative or qualitative use in risk assessment because it is of insufficient quality and lacks scientific defensibility)	- This study is concerned with the effect of a glyphosate-tolerant soybean-based diet compared to a conventional soybean-based diet during development and maturation in mice. It does not deal with the effect of glyphosate itself as an active ingredient. - While the transgenic soybean crop was treated with glyphosate there is no indication that residues of glyphosate remain, nor are there any details of the actual crop treatment.
Reproductive toxicity studies with octamethyl cyclotetrasiloxane in female rats using various exposure regimens. (Ref. No. 49)	NA	Invalid (inappropriate for quantitative or qualitative use in risk assessment)	Unacceptable (inappropriate for quantitative or qualitative use in risk assessment because it is of insufficient quality and lacks scientific defensibility)	- This study does not deal with the active ingredient glyphosate. - The report is an abstract of a conference poster.
Effect of the herbicide glyphosate on enzymatic activity in pregnant rats and their foetuses. (Ref. No. 19)	NA	Invalid (inappropriate for quantitative or qualitative use in risk assessment)	Unacceptable (inappropriate for quantitative or qualitative use in risk assessment because it is of insufficient quality and lacks scientific defensibility)	- The test substance was a glyphosate-based commercial product. - The percent active ingredient and purity were not stated/measured. - Only two dose groups were used.
The teratogenic potential of the herbicide glyphosate-Roundup in Wistar rats. (Ref. No. 17)	NA	Invalid (inappropriate for quantitative or qualitative use in risk assessment)	Unacceptable (inappropriate for quantitative or qualitative use in risk assessment because it is of insufficient quality and lacks scientific defensibility)	- The test substance was a glyphosate based commercial formulation. - The active ingredient was not measured - No information was provided on the inert ingredients.
The impact of simultaneous intoxication with agrochemicals on the antioxidant defense system in rat. (Ref. No. 4)	NA	Invalid (inappropriate for quantitative or qualitative use in risk assessment)	Unacceptable (inappropriate for quantitative or qualitative use in risk assessment because it is of insufficient quality and lacks scientific defensibility)	- The percent active ingredient and purity were not stated for the individual pesticides. - The route of exposure was via i.p. injection, which is not relevant to human health.

Table A-1. Glyphosate Literature Search with PMRA — Primary and Secondary Review Findings				
Study Title (Reference # in Appendix B)	**Study Type**	**Primary Review (PMRA)**	**Secondary Review (U.S. EPA)**	**Limitations/Deficiencies[2]**
				- A single dose level for each pesticide was compared to the control. All other treatments involved combinations of more than one pesticide.
Oxidative stress responses of rats exposed to Roundup and its active ingredient glyphosate. (Ref. No. 22)	NA	Invalid (inappropriate for quantitative or qualitative use in risk assessment)	Unacceptable (inappropriate for quantitative or qualitative use in risk assessment because it is of insufficient quality and lacks scientific defensibility)	- Amount of active ingredient during exposure was not measured. - The route of exposure was via i.p. injection, which is not relevant to human health. - A single dose level for each pesticide was compared to the control. All other treatments involved combinations of more than one pesticide.
Studies on glyphosate-induced carcinogenicity in mouse skin: a proteomic approach. (Ref. No. 26)	NA	Invalid (inappropriate for quantitative or qualitative use in risk assessment)	Unacceptable (inappropriate for quantitative or qualitative use in risk assessment because it is of insufficient quality and lacks scientific defensibility)	- The test substance was a glyphosate based commercial formulation and the active ingredient was not measured. - No information was provided on the inert ingredients.
Genotoxic potential of glyphosate formulations: mode-of-action investigations. (Ref. No. 28)	NA	Invalid (inappropriate for quantitative or qualitative use in risk assessment)	Unacceptable (inappropriate for quantitative or qualitative use in risk assessment because it is of insufficient quality and lacks scientific defensibility)	- The test substance was a glyphosate based commercial formulation. - No information was provided on the inert ingredients. - The percent active ingredient and purity were not stated/measured for the individual pesticides. - The study was largely concerned with i.p. injection route of exposure, which is not relevant to human health.
The effect of sub-acute and sub-chronic exposure of rats to the glyphosate-based herbicide Roundup. (Ref. No. 14)	NA	Invalid (inappropriate for quantitative or qualitative use in risk assessment)	Unacceptable (inappropriate for quantitative or qualitative use in risk assessment because it is of insufficient quality and lacks scientific defensibility)	- The test substance was a glyphosate-based commercial formulation. - No information was provided on the inert ingredients. - A number of important experimental details are not included (i.e., number of animals/group, method of administering dose, etc.) - Discussion of results is vague with references to a variety of other experiments rather than the results of the present study. - Very few statistically significant effects occur. Effects indicated as “mild effects” or “mild differences” do not appear to be statistically significant.

Table A-1. Glyphosate Literature Search with PMRA — Primary and Secondary Review Findings				
Study Title (Reference # in Appendix B)	**Study Type**	**Primary Review (PMRA)**	**Secondary Review (U.S. EPA)**	**Limitations/Deficiencies[2]**
The effects of sub-chronic exposure of Wistar rats to the herbicide Glyphosate-Biocarb®. (Ref. No. 8)	NA	Invalid (inappropriate for quantitative or qualitative use in risk assessment)	Unacceptable (inappropriate for quantitative or qualitative use in risk assessment because it is of insufficient quality and lacks scientific defensibility)	- The test substance was a glyphosate based commercial formulation and the active ingredient was not measured. - No information was provided on the inert ingredients.
Clastogenic effects of glyphosate in bone marrow cells of Swiss albino mice. (Ref. No. 47)	NA	Invalid (inappropriate for quantitative or qualitative use in risk assessment)	Unacceptable (inappropriate for quantitative or qualitative use in risk assessment because it is of insufficient quality and lacks scientific defensibility)	- The test substance was a glyphosate based commercial formulation and the active ingredient was not measured. - No information was provided on the inert ingredients. - The route of exposure was via i.p. injection, which is not relevant to human health.
Evaluation of genome damage and its relation to oxidative stress induced by glyphosate in human lymphocytes in vitro. (Ref. No. 41)	NA	Invalid (inappropriate for quantitative or qualitative use in risk assessment)	Unacceptable (inappropriate for quantitative or qualitative use in risk assessment because it is of insufficient quality and lacks scientific defensibility)	- Exposure was directly to blood samples obtained from human volunteers. - The authors concede that the lack of statistical significance at lower concentrations may be due to the low number of samples included in the study.
Time- and dose-dependent effects of Roundup on human embryonic and placental cells. (Ref. No. 6)	NA	Invalid (inappropriate for quantitative or qualitative use in risk assessment)	Unacceptable (inappropriate for quantitative or qualitative use in risk assessment because it is of insufficient quality and lacks scientific defensibility)	- Exposure was directly to human embryonic and placental cells as well as other tissues. - The active ingredient was not measured. - It is difficult to extrapolate in vitro effects with in vivo toxicity. - The percent purity of the reagent grade glyphosate was not stated.
Exposure to pesticides increases levels of uPA and uPAR in pre-malignant human prostate cells. (Ref. No. 46)	NA	Invalid (inappropriate for quantitative or qualitative use in risk assessment)	Unacceptable (inappropriate for quantitative or qualitative use in risk assessment because it is of insufficient quality and lacks scientific defensibility)	- It is difficult to extrapolate in vitro effects with in vivo toxicity. - The percent purity of the reagent grade glyphosate was not stated. - For the glyphosate based commercial formulation used, the percent active ingredient and purity was not indicated/measured and other components were not identified.
Oral bioavailability of glyphosate: studies using two intestinal cell lines. (Ref. No. 56)	NA	Invalid (inappropriate for quantitative or qualitative use in risk assessment)	Unacceptable (inappropriate for quantitative or qualitative use in risk assessment because it is of insufficient quality and lacks scientific defensibility)	- Exposure was directly to the intestinal epithelial barrier using two cell lines. - The applicability to in vivo human risk is unclear. - The percent purity of the reagent grade glyphosate is given as approximately 95% pure (not validated).
Cysteine turnover in human cell lines is influenced by glyphosate. (Ref. No. 30)	NA	Invalid (inappropriate for quantitative or qualitative use in risk assessment)	Unacceptable (inappropriate for quantitative or qualitative use in risk assessment because it is of	- Exposure was directly to human cell lines. - The percent purity of the reagent grade glyphosate is not stated

Table A-1. Glyphosate Literature Search with PMRA — Primary and Secondary Review Findings				
Study Title (Reference # in Appendix B)	**Study Type**	**Primary Review (PMRA)**	**Secondary Review (U.S. EPA)**	**Limitations/Deficiencies[2]**
			insufficient quality and lacks scientific defensibility)	- The active ingredient was not measured. - The number of glyphosate doses (2) was limited.
The hemodynamic effects of the formulation of glyphosate-surfactant herbicides. (Ref. No. 33)	NA	Invalid (inappropriate for quantitative or qualitative use in risk assessment because it is of insufficient quality and lacks scientific defensibility)	Unacceptable (inappropriate for quantitative or qualitative use in risk assessment because it is of insufficient quality and lacks scientific defensibility)	- The test substance was a glyphosate based commercial formulation. - The percent active ingredient and purity of the test substances for exposure were not indicated/measured and other components were not identified. - Only a single dose of each test substance was utilized. - Pigs are atypical animals used in toxicology studies reviewed by regulatory agencies.
Genotoxicity of AMPA, the environmental metabolite of glyphosate, assessed by the Comet assay and cytogenetics tests. (Ref. No. 36)	NA	Invalid (inappropriate for quantitative or qualitative use in risk assessment because it is of insufficient quality and lacks scientific defensibility)	Unacceptable (inappropriate for quantitative or qualitative use in risk assessment because it is of insufficient quality and lacks scientific defensibility)	- The test substance was the primary metabolite of glyphosate, not the active ingredient. - Analytical measurements for exposure were not provided.
Ethoxylated adjuvants of glyphosate-based herbicides are active principles of human cell toxicity. (Ref. No. 39)	NA	Invalid (inappropriate for quantitative or qualitative use in risk assessment because it is of insufficient quality and lacks scientific defensibility)	Unacceptable (inappropriate for quantitative or qualitative use in risk assessment because it is of insufficient quality and lacks scientific defensibility)	- Purity of glyphosate was not specified. - Only a single concentration was used as a control. - For glyphosate based commercial formulations, the percent active ingredient and purity of the test substances were not indicated and other components were not identified. Analytical measurements for exposure were not provided. - Data is mainly presented in graph format (no raw data provided), making evaluation difficult. - Data for LC50 for glyphosate in HepG2 cell line was not determined. - It is difficult to extrapolate in vitro effects with in vivo toxicity.
Comparative effects of the Roundup and glyphosate on mitochandrial oxidative phosphorylation. (Ref. No. 44)	NA	Invalid (inappropriate for quantitative or qualitative use in risk assessment because it is of insufficient quality and lacks scientific defensibility)	Unacceptable (inappropriate for quantitative or qualitative use in risk assessment because it is of insufficient quality and lacks scientific defensibility)	- The test substance was glyphosate and a glyphosate-based commercial formulation. - The percent active ingredient and purity of glyphosate and the formulation were not indicated/measured and other components of the formulation were not identified.
Comparison of the effect of Roundup Ultra 360 SL pesticide and its active compound glyphosate on human erythrocytes. (Ref. No. 45)	NA	Invalid (inappropriate for quantitative or qualitative use in risk assessment because it is of	Unacceptable (inappropriate for quantitative or qualitative use in risk assessment because it is of	- Exposure directly to human blood in vitro of commercial formulation. - Analytical measurements for exposure were not provided.

Table A-1. Glyphosate Literature Search with PMRA — Primary and Secondary Review Findings				
Study Title (Reference # in Appendix B)	**Study Type**	**Primary Review (PMRA)**	**Secondary Review (U.S. EPA)**	**Limitations/Deficiencies[2]**
		insufficient quality and lacks scientific defensibility)	insufficient quality and lacks scientific defensibility)	- Provided poor description of blood donors. - It is difficult to extrapolate in vitro effects with in vivo toxicity. - A positive control was not included. - pH measurement was not included. - Other components of the formulation were not identified.
Genotoxicity testing of the herbicide Roundup and its active ingredient glyphosate isopropylamine using the mouse bone marrow micronucleus test, Salmonella mutagenicity test and Allium anaphase-telophase test. (Ref. No. 48)	NA	Invalid (inappropriate for quantitative or qualitative use in risk assessment because it is of insufficient quality and lacks scientific defensibility)	Unacceptable (inappropriate for quantitative or qualitative use in risk assessment because it is of insufficient quality and lacks scientific defensibility)	- The percent active ingredient and purity of the glyphosate based commercial formulation was not indicated/measured and other components were not identified. - Cytotoxicity/cell survival was not measured. - A greater number of bacterial strains needed to be used.
Differential effects of glyphosate and Roundup on human placental cells and aromatase. (Ref. No. 50)	NA	Invalid (inappropriate for quantitative or qualitative use in risk assessment because it is of insufficient quality and lacks scientific defensibility)	Unacceptable (inappropriate for quantitative or qualitative use in risk assessment because it is of insufficient quality and lacks scientific defensibility)	- The test substances were pure technical glyphosate and a glyphosate commercial formulation. - The purity of glyphosate was not specified. - The percent active ingredient and purity of the glyphosate based commercial formulation was not indicated and other components were not identified.
Glyphosate and AMPA in drinking water. (Ref. No. 61)	NA	Invalid (inappropriate for quantitative or qualitative use in risk assessment because it is of insufficient quality and lacks scientific defensibility)	Unacceptable (inappropriate for quantitative or qualitative use in risk assessment because it is of insufficient quality and lacks scientific defensibility)	- Reference only included summaries of studies. - Reproductive studies with glyphosate included only limited histopathological examination. - Long term studies and reproductive toxicity studies for primary metabolite, AMPA are unavailable.
Safety evaluation and risk assessment of the herbicide roundup and its active ingredient, glyphosate, for humans. (Ref. No. 59)	NA	Invalid (inappropriate for quantitative or qualitative use in risk assessment because it is of insufficient quality and lacks scientific defensibility)	Unacceptable (inappropriate for quantitative or qualitative use in risk assessment because it is of insufficient quality and lacks scientific defensibility)	- Reference is for a review paper, not a single study.
Developmental and reproductive outcomes in humans and animals after glyphosate exposure: a critical analysis. (Ref. No. 60)	NA	Invalid (inappropriate for quantitative or qualitative use in risk assessment because it is of insufficient quality and lacks scientific defensibility)	Unacceptable (inappropriate for quantitative or qualitative use in risk assessment because it is of insufficient quality and lacks scientific defensibility)	- Reference is for a review paper, not a single study. - Most human studies involved glyphosate based commercial formulations, and have poor characterization of the test substance. - Some studies suffered from numerous inadequacies in design and reporting.

NA = not applicable. Study does not correspond with any guideline studies.

[2]Listed are some of the limitations/deficiencies noted during preliminary review that indicated the study would be considered unacceptable for use in risk assessment. These do not represent all potential limitations/deficiencies.

Appendix B: Studies included in the joint review with PMRA

1. Acquavella, J. F., Alexander, B. H., Mandel, J. S., Gustin, C., Baker, B., Chapman, P., and Bleeke, M. 2004. Glyphosate biomonitoring for farmers and their families: results from the Farm Family Exposure Study. Environmental Health Perspectives. 112(3):321-326.

2. Adam, A., Marzuki, A., Abdul Rahman, H. and Abdul Aziz, M. 1997. The oral and intratracheal toxicities of Roundup and its components to rats. Vet Human Toxicol. 39: 147-51.

3. Anadon, A., Martinez-Larranaga, M.R., Martinez, M.A., Castellano, V.J., Martinez, M., Martin, M.T., Nozal, M.J., and Bernal, J.L. 2009. Toxicokinetics of glyphosate and its metabolite aminomethyl phosphonic acid in rats. Toxicology Letters. 190: 91-95.

4. Astiz, M., de Alaniz, M.J.T., and Marra, C.A. 2009. The impact of simultaneous intoxication with agrochemicals on the antioxidant defense system in rat. Pesticide Biochemistry and Physiology 94:93-99.

5. Arbuckle, T. E., Lin, S. and Mery, L. S. 2001. An explanatory analysis of the effect of pesticide exposure on the risk of spontaneous abortion in an Ontario farm population. Environmental Health Perspectives. 109 (3): 851-857.

6. Benachour, N., Sipahutar, H., Moslemi, S., Gasnier, C., Travert, C., and Séralini, G.E. 2007. Time- and dose-dependent effects of Roundup on human embryonic and placental cells. Archives of Environmental Contamination and Toxicology. 53(1): 126-133.

7. Benachour, N. and Séralini, G. 2009. Glyphosate Formulations Induce Apoptosis and Necrosis in Human Umbilical, Embryonic, and Placental Cells. Chem. Res. 22 (1), pp 97–105.

8. Benedetti, A.L., Vituri, C.de L., Trentin, A.G., Domingues, M.A.C.D., and Alvarez-Silva, M.. 2004. The effects of sub-chronic exposure of Wistar rats to the herbicide Glyphosate-Biocarb®. Toxicol. Lett. 153(2):227-232.

9. Beuret, C.J., Zirulnik, F, and Giménez, M.S. 2005. Effect of the herbicide glyphosate on liver lipoperoxidation in pregnant rats and their foetuses. Reproductive Toxicology 19:501-504.

10. Brake, D.G. and Evenson, D.P. 2004. A generational study of glyphosate-tolerant soybeans on mouse fetal, postnatal, pubertal and adult testicular development. Food Chem. Toxicol. 42(1):29-36.

11. Bolognesi, C., Carrasquilla, Volpi, S., Solomon, K.R., and Marshall, E.J.P. 2009. Biomonitoring of genotoxic risk in agricultural workers from five Columbian regions: association to occupational exposure to glyphosate. Journal of toxicology and Environmental Health, Part A: Current issues, 72:15-16, 989-997.

12. Bonn, D. 2005. Roundup revelation: weed killer adjuvants may boost toxicity. International health Perspectives 13(6): A403-404.

13. Bradberry SM, Proudfoot AT, Vale JA. 2004. Glyphosate poisoning. Toxicol Rev. 23(3):159-67.

14. Çağlar, S. and Kolankaya, D. 2008. The effect of sub-acute and sub-chronic exposure of rats to the glyphosate-based herbicide Roundup. Environ. Toxicol. Pharmacol. 25(1):57-62.

15. Chang, C. B., and Chang, C. C. 2009. Refractory cardiopulmonary failure after glyphosate surfactant intoxication: a case report. J Occup Med Toxicol. 4:2.

16. Clair, É., Mesnage, R., Travert, C., and Séralini, G. É. 2012. A glyphosate-based herbicide induces necrosis and apoptosis in mature rat testicular cells in vitro, and testosterone decrease at lower levels. Toxicology in Vitro,26(2):269-279.

17. Dallegrave, E., Mantese, F.D., Coelho, R.S., Pereira, J.D., Dalsenter, P.R. & Langeloh, A. 2003. The teratogenic potential of the herbicide glyphosate-Roundup in Wistar rats. Toxicology Letters. 142:45-52.

18. Dallegrave, E., Mantese, F.D., Oliveira, R.T., Andrade, A.J.M., Dalsenter, P.R., and Langeloh, A. 2007. Pre- and postnatal toxicity of the commercial glyphosate formulation in Wistar rats. Archives of Toxicology. 81 (9): 665-673.

19. Daruich, J., Zirulnick, F., & Giménez, M.S. 2001. Effect of the herbicide glyphosate on enzymatic activity in pregnant rats and their foetuses. Environmental Research. 85:226-231.

20. De Roos, A.J., Blair, A., Rusiecki, A.A., Hoppin, J.A., Svec, M., Dosemeci, M., Sandler, D.P., and Alavanja, M.C. 2004. Cancer incidence among glyphosate-exposed pesticide applicators in the Agricultural Health Study. Environmental Health Perspectives, 113(1): 49-54.

21. Elie-Caille, C., Heu, C., Guyon, C. and Nicod, L. 2010. Morphological damages of a glyphosate-treated keratinocyte cell line revealed by a micro- to nanoscale microscopic investigation. Cell Biol Toxicol. 26: 331-339.

22. El-Shenawy, N.S. 2009. Oxidative stress responses of rats exposed to Roundup and its active ingredient glyphosate. Environ. Toxicol. Pharmacol. 28(3):379-385.

23. Gasnier, C., Dumont, C., Benachour, N., Clair, E. Chagnon, M., Seralini, G. 2009. Glyphosate-based herbicides are toxic and endocrine disruptors in human cell lines. Toxicology. 262(3): 184-191.

24. Gasnier, C., Benachour, N., Clair, E., Travert, C., Langlois, F., Laurent, C., Decroix-Laporte, C., and Seralini, G. 2010. Dig 1 protects against cell death provoked by glyphosate-based herbicides in human liver cell lines. Journal of Occupational Medicine and Toxicology. 5:29.

25. Gehin, A., Guillaume, Y. C., Millet, J., Guyon, C., and Nicod, L. 2005. Vitamins C and E reverse effect of herbicide-induced toxicity on human epidermal cells HaCaT: a biochemometric approach. International journal of pharmaceutics. 288(2):219-226.

26. George, J., Prasad, S., Mahmood, Z. & Shukla, Y. 2010. Studies on glyphosate-induced carcinogenicity in mouse skin: a proteomic approach. J. Proteomics. 73(5):951-964.

27. Goldstein DA, Farmer DL, Levine SL, Garnett RP. 2005. Mechanism of Toxicity of Commercial Glyphosate Formulatons: How Important is the Surfactant? Journal of Toxicology: Clinical Toxicology, 43(5):423-424.

28. Heydens, W.F., Healy, C.E., Hotz, K.J., Kier, L.D., Martens, M.A., Wilson, A.G.E., and Farmer, D.R. 2008. Genotoxic potential of glyphosate formulations: mode-of-action investigations. J. Agric. Food Chem. 56(4):1517-1523.

29. Hokanson, R., Fudge, R., Chowdhary, R., and Busbee, D. 2007. Alteration of estrogen-regulated gene expression in human cells induced by the agricultural and horticultural herbicide glyphosate. Human & experimental toxicology. 26(9):747-752.

30. Hultberg, M. (2007) Cysteine turnover in human cell lines is influenced by glyphosate. Environ. Toxicol. Pharmacol. 24(1):19-22.

31. Lee, H. L., Chen, K. W., Chi, C. H., Huang, J. J., and Tsai, L. M.. 2000. Clinical Presentations and Prognostic Factors of a Glyphosate — Surfactant Herbicide Intoxication: A Review of 131 Cases. Academic Emergency Medicine. 7(8):906-10.

32. Lee, C. H., Shih, C. P., Hsu, K. H., Hung, D. Z., & Lin, C. C. 2008.. The early prognostic factors of glyphosate-surfactant intoxication. American Journal of Emergency Medicine 26(3): 275-281.

33. Lee, H.L. and Guo, H.R. 2011. The Hemodynamic Effects of the Formulation of Glyphosate-Surfactant Herbicides. Herbicides, Theory and Applications Prof. M Larramendy (Ed.) ISBN, 978-953.

34. Li, A.P. and Long, T.J. 1988. An evaluation of the genotoxic potential of glyphosate. Fundamental and Applied Toxicology 10:537-546. Institute of Environmental Toxicology, Tokyo.

35. Malhotra R.C., Ghia, D.K., Cordato, D. J. and Beran, R.G. 2010. Glyphosate-surfactant herbicide-induced reversible encephalopathy. Journal of Clinical Neuroscience. 17:1472-1473.

36. Manas, F., Peralta, L. Raviolo, J., Garcıa Ovando, H., Weyers, A., Ugnia, L., GonzalezCid, M., Larripa, I., Gorla, N. 2009. Genotoxicity of AMPA, the environmental metabolite of glyphosate, assessed by the Comet assay and cytogenetic tests. Ecotoxicology and Environmental Safety. 72: 834-837.

37. Marc, J., Mulner-Lorillon, O., and Bellé, R. 2004. Glyphosate-based pesticides affect cell cycle regulation. Biology of the Cell. 96(3):245-249.

38. McQueen, H., Callan, A. C., and Hinwood, A. L. 2012. Estimating maternal and prenatal exposure to glyphosate in the community setting. International journal of hygiene and

environmental health, 215(6):570-576.

39. Mesnage, R., Bernay, B., and Seralini, G. 2013. Ethoxylated adjuvants of glyphosate-based herbicides are active principles of human cell toxicity. Toxicology. 313: 122-128.

40. Mink, P.J., Mandel, J.S., Lundin, J.I. and Sceurman, B.K. 2011. Epidemiologic studies of glyphosate and non-cancer health outcomes: a review. Regulatory Toxicology and Pharmacology. 61:172-184.

41. Mladinic, M., Berend, S., Vrdoljak, A.L., Kopjar, N. Radic, B. and Zeljezic, D. 2009. Evaluation of genome damage and its relation to oxidative stress induced by glyphosate in human lymphocytes in vitro. Environ. Mol. Mutagen. 50(9): 800-807.

42. Mose, T., Kjaerstad, M. B., Mathiesen, L., Nielsen, J. B., Edelfors, S., and Knudsen, L. E. 2008. Placental passage of benzoic acid, caffeine, and glyphosate in an ex vivo human perfusion system. Journal of Toxicology and Environmental Health, Part A. 71(15):984-991.

43. NTP Technical Report on Toxicity Studies of Glyphosate Administered in Dosed Feed to F344/N Rats and B6C3F1 Mice. 1992. Glyphosate, NTP Toxicity Report Number 16.

44. Peixoto, F. 2005. Comparative effects of the Roundup and glyphosate on mitochondrial oxidative phosphorylation. Chemosphere 61:1115-1122.

45. Pieniazek, D., Burkowska, B. and Duda, W. 2004. Comparison of the effect of Roundup Ultra 360 SL pesticide and its active compound glyphosate on human erythrocytes. Pesticide Biochemistry and Physiology. 79:58-63.

46. Potti, A. and Seghal, I. 2005. Exposure to pesticides increases levels of uPA and uPAR in pre-malignant human prostate cells. Environ. Toxicol. Pharmacol. 19(2): 215-219.

47. Prasad, S., Srivasta, S., Singh, M. and Shukla, Y. 2009. Clastogenic effects of glyphosate in bone marrow cells of Swiss albino mice. J. Toxicol. 2009: 6 pages.

48. Rank, J., Jensen, A.-G., Skov, B., Pendersen, L.H. and Jensen, K. 1993. Genotoxicity testing of the herbicide Roundup and its active ingredient glyphosate isopropylamine using the mouse bone marrow micronucleous test, Salmonella mutagenicity test, and Allium anaphase-telophase test. Mutation Research. 300:29-36.

49. Reynolds, V.L., DeSesso, J.M., Mast, R.W., Stump, D.G. and Holson, J.F. 1998. Reproductive toxicity studies with octamethyl cyclotetrasiloxane in female rats using various exposure regimens. Toxicology Letters 95: 216

50. Richard, S., Moslemi, S., Sipahutar, H., Benachour, N. and Seralinin, G-E. 2005. Differential effects of glyphosate and Roundup on human placental cells and aromatase. Environmental Health Perspectives. 113(6): 716-720.

51. Roberts, D. M., Buckley, N. A., Mohamed, F., Eddleston, M., Goldstein, D. A., Mehrsheikh,

A., Bleeke, M.S., and Dawson, A. H. 2010. A prospective observational study of the clinical toxicology of glyphosate-containing herbicides in adults with acute self-poisoning. Clinical toxicology, 48(2):129-136.

52. Romano, R.M., Romano, M.A., Bernardi, M.M., Furtado, P.V. and Oliveira, C.A. 2010. Prepubertal exposure to commercial formulation of the herbicide glyphosate alters testosterone levels and testicular morphology. Arch. Toxicol. 84(4):309-317.

53. Romano, M.A. 2012. Glyphosate impairs male offspring reproductive development by disrupting gonadotropin expression. Arch. Toxicol. 86:663-673.

54. Stella, J. and Ryan, M. 2004. Herbicide formulation: A potentially lethal ingestion. Emergency Medicine Australasia. 16: 235-239.

55. Talbot, A.R., Shiaw, M.H., Huang, J.S., Yang, S.F., Goo, T.S., Wang, S.H., and Sanford, T.R. 1991. Acute poisoning with a glyphosate-surfactant herbicide ('roundup'): a review of 93 cases. Human and Experimental Toxicology. 10(1):1-8.

56. Vasiluk, L., Pinto, L.J. and Moore, M.M. 2005. Oral bioavailability of glyphosate: studies using two intestinal cell lines. Environ. Toxicol. Chem. 24(1):153-160.

57. Walsh, L.P., McCormick, C., Martin, C., and Stocco, D.M. 2000. Roundup Inhibits Steroidogenesis by Disrupting Steroidogenic Acute Regulatory (StAR) Protein Expression. Environ Health Perspect. 108(8): 769–776.

58. Wang, G., Fan, X. N., Tan, Y. Y., Cheng, Q., & Chen, S. D. 2011. Parkinsonism after chronic occupational exposure to glyphosate. Parkinsonism & Related Disorders. 17(6):486-487.

59. Williams, G.M., Kroes, R., and Munro, I.C. 1999. Safety Evaluation and Risk Assessment of the Herbicide Roundup and Its Active Ingredient, Glyphosate, for Humans. Regulatory Toxicology and Pharmacology. 31(2): 117-165.

60. Williams, A.L., Watson, R.E., and DeSesso, J.M. 2012. Developmental and Reproductive Outcomes in Humans and Animals After Glyphosate Exposure: A Critical Analysis. Journal of Toxicology and Environmental Health, Part B: Critical Reviews. 15:1, 39-96

61. World Health Organization. 2005. Glyphosate and AMPA in drinking water. Background document for development of WHO Guidelines for Drinking-water Quality. WHO/SDE/WSH/03.04/97.

62. Wu JY, Chang SS, Tseng CP, Deng JF, Lee CC. 2006. Parenteral glyphosate-surfactant herbicide intoxication. Am J Emerg Med. 24(4):504-6.

Appendix C: Data Evaluation Records (DERs) generated for joint review with PMRA

Note: In studies classified acceptable and appropriate for quantitative use, PMRA and EPA did not agree on all of the selected NOAEL/LOAEL values. Additionally, DERs are not presented for the 17 studies related to poisoning/incidents, biomonitoring data, and epidemiological studies that were not included as part of this review since they were evaluated as part of the glyphosate Tier II incident report.

Reviewer # 1912 **Date** July 10, 2012

Study Type: Short-Term Oral (90-day) (rodent) [feeding] - Rat; OPPTS 870.3100 (rodent); OECD 408.

Test Material (purity): Glyphosate (99% a.i.)

Synonyms: Glyphosate, technical grade; Glycine, N-(phosphonomethyl); N-phosphono-methyl glycine; N-(phosphonomethyl)glycine; MON0573; MON 2139.

Citation: NTP Technical Report on Toxicity Studies of Glyphosate Administered in Dosed Feed to F344/N Rats and B6C3F1 Mice. Laboratory name: Southern Research Institute, Birmingham, AL. Laboratory report number: Glyphosate, NTP Toxicity Report Number 16. Study report date: July 1992.

Sponsor: None

MRID: N/A

Executive Summary: In a short term chronic toxicity study, glyphosate (99%) was administered to 10 F344/N Rats/sex/group at dietary concentrations of 0, 3125, 6250, 12500, 25000, or 50000 ppm (equivalent to 0, 205, 410, 811, 1678, or 3393 mg/kg bw/day for males and 0, 213, 421, 844, 1690 or 3393 mg/kg bw/day for females, respectively) for 90 days. Ten additional rats/sex were included at each dietary level for evaluation of hematology and clinical chemistry parameters.

All animals survived to necropsy. Diarrhea was noted in the high dose animals. Body weight and body weight gain were reduced in the two high doses in males and high dose in females.

Mild increases in RBC counts, hematocrit, and hemoglobin concentrations were noted in males, mostly in the three high doses. In high dose females, slight increases in lymphocyte and platelet counts, WBC, MCH, and MCV were noted.

Increase in the activities of ALP in the treated animals was noted. In addition, increased relative liver weights in the two high doses and ALT in four high doses were noted in males. These findings are consistent of a hepatobiliary effect that can be attributed to glyphosate administration.

A decrease in absolute and relative thymus weight was noted across all dose groups. Absolute heart weight was increased in the high dose animals. Relative right kidney and right testis weights were increased in the two high dose male groups. In high dose females, relative right kidney weight was increased.

A dose-related increase in the incidence and severity of cytoplasmic alteration of the parotid and submandibular salivary glands was noted in the microscopic evaluations.

A decrease in sperm counts was noted in the three high doses. Longer estrous cycle was noted in the high dose group. No other treatment related finding was noted in the evaluations of reproductive tissues.

PMRA: The LOAEL is 205/213 mg/kg bw/day for males/females based on dose related increase in the incidence and severity of cytoplasmic alterations in the parotid and submandibular salivary glands of male and female rats. A NOAEL was not determined.

EPA: The NOAEL is 410 and 421 mg/kg/day for males and females, respectively. The LOAEL is 811/844 mg/kg/day based on mild cytoplasmic alterations in the parotid and submandibular salivary glands. The alterations at lower doses were scored as minimal in severity and not considered adverse.

Although this study was not conducted according the guideline requirement for a subchronic oral study (OPPTS 870.3100; OECD 408), it is considered acceptable and classified as appropriate for quantitative use for hazard characterization of glyphosate.

Compliance: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were not provided. However, this study was peer-reviewed by NTP internally. The peer-review panel determined that the design and conditions of this study was appropriate and ensured that the toxicity study report presented the experimental results and conclusions thoroughly and clearly.

I. MATERIALS AND METHODS

A. Materials

Test material:	Glyphosate	
Description:	White solid, stable for at least 3 weeks in room temperature in the dark	
Lot/Batch #:	Obtained from Monsanto Agricultural Products (St. Louis)	
Purity:	99 % a.i.	
CAS #:	1071-83-6	
Vehicle:	None	
Positive control:	None	
Test species:	Rat	
Strain:	F344/N	
Age at start:	43 days of age	
Weight at start:	♂: 108-115g □♀:92-95g □	
Source:	Simonsen Laboratories (Gilroy, CA, USA)	
Housing:	5 per cage	
Diet:	NIH-07 feed was provided *ad libitum*	
Water:	NIH-07 water was provided *ad libitum*	
Environmental conditions:	**Temperature:**	67-74°F
	Humidity:	40-89%
	Air changes:	10/hr
	Photoperiod:	12 hours dark/12 hours light
Acclimation:	12 days	

B. Study design and methods

1. Study experimentation dates - Start: May -1988 End: September - 1988

2. Animal assignment - Animals were assigned randomly (stratified weight method first and then assigned to study groups in a random order) to the test groups noted in Table 1. The NTP used copies of proprietary reports of toxicity studies performed by Monsanto Corporation in designing its glyphosate studies.

Table 1: Study Design[a]

Dose (mg/kg bw/d)		Conc. in Diet (ppm)	Number of Animals Main Study - 3 months		Hematology and Clinical Chemistry Groups	
Male	**Female**		**Male**	**Female**	**Male**	**Female**
0	**0**	0	10	10	10	10
205	213	3125	10	10	10	10
410	421	6250	10	10	10	10
811	844	12500	10	10	10	10
1678	1690	25000	10	10	10	10
3393	3393	50000	10	10	10	10

[a] Data were extracted from page 12 of the study report

3. Diet preparation and analysis - The report stated that glyphosate when mixed with diet and stored at room temperature in the dark, was stable for at least 3 weeks. Further details and data were not provided in the report.

4. Statistics -

"Two approaches were employed to assess the significance of pairwise comparisons between dosed and control groups in the analysis of continuous variables. Organ and body weight data, which are approximately normally distributed, were analyzed using the parametric multiple comparisons procedures of Williams (1971, 1972, 1986) and Dunnett (1955). Clinical pathology and hematology data, which typically have skewed distributions were analyzed using nonparametric multiple comparisons methods of Shirley (1977) and Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of dose-response trends and to determine whether a trend-sensitive test (Williams, Shirely) was more appropriate for pairwise comparisons than a test capable of detecting departures from monotonic dose response (Dunnett, Dunn). If the P-value from Jonchkheere's test was greater than or equal to 0.10, Dunn's or Dunnett's test was used rather than Shirley's or Williams's test. The outlier test of Dixon and Massey (1951) was employed to detect extreme values. No value selected by the outlier test was eliminated unless it was at least twice the next largest value or at most half of the next smallest value.

Analysis of Vaginal Cytology Data

Since the data are proportions (the proportion of the observation period that an animal was in a given estrous state), an arcsine transformation was used to bring the data into closer conformance with normality assumptions. Treatment effects were investigated by applying a multivariate analysis of variance (Morrison, 1976) to the transformed data to test for the simultaneous equality of measurements across dose levels."

C. Methods

1. Observations - Animals were inspected for mortality/moribundity (twice daily), and clinical signs of toxicity (once a week).

2. Body weight - Animals were weighed weekly.

3. Food consumption and compound intake - Food consumption for each animal was determined as average food consumption in g/animal/day. Compound intake (mg/kg bw/day) values were estimated as time-weighted averages from the consumption and body weight gain data.

4. Hematology and clinical chemistry - For clinical pathology studies, animals were anesthetized with a mixture of carbon dioxide and oxygen (70%:30%), and blood samples were collected from the retro-orbital sinus using heparinized microcapillary tubes. The checked (X) parameters were examined.

a. Hematology

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)*
X	Leukocyte count (WBC)*	X	Mean corpuscular HGB concentration (MCHC)*
X	Erythrocyte count (RBC)*	X	Mean corpuscular volume (MCV)*
X	Platelet count*		Reticulocyte count
	Blood clotting measurements*		
	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

* Recommended for subchronic rodent studies based on Guideline 870.3100

b. Clinical chemistry

	ELECTROLYTES		OTHER
	Calcium*	X	Albumin*
	Chloride*	X	Blood creatinine*
	Magnesium	X	Blood urea nitrogen*
	Phosphorus*		Total Cholesterol*
	Potassium*		Globulins
	Sodium*		Glucose*
	ENZYMES		Total bilirubin
X	Alkaline phosphatase (ALP)	X	Total serum protein (TP)*
	Cholinesterase (ChE)		Triglycerides
X	Creatine phosphokinase		Serum protein electrophores
	Lactic acid dehydrogenase (LDH)	X	Total bile acids
X	Serum alanine amino-transferase (ALT/also SGPT)*		
	Serum aspartate amino-transferase (AST/also SGOT)*		
X	Sorbitol dehydrogenase		
	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

* Recommended for subchronic rodent studies based on Guideline 870.3100

5. <u>Sacrifice and pathology</u> - All animals that died and those sacrificed on schedule were subjected to gross pathological examination. The checked (X) tissues were collected for histological examination from the control and high dose group animals. The salivary gland was examined in all dose groups. In addition, the (XX) organs were weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
	Tongue		Aorta*	X	Brain*+
X	Salivary glands*	XX	Heart*+		Peripheral nerve*
X	Esophagus*		Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	X	Pituitary*
X	Duodenum*	X	Spleen*+		Eyes (optic nerve)*
X	Jejunum*	XX	Thymus*+		**GLANDULAR**
X	Ileum*			X	Adrenal gland*+
X	Cecum*		**UROGENITAL**		Lacrimal gland[T]
X	Colon*	XX	Kidneys*+	X	Mammary gland*
X	Rectum*	X	Urinary bladder*	X	Parathyroid*
XX	Liver*+	XX	Testes*+	X	Thyroid*
X	Pancreas*	X	Epididymides*+		**OTHER**
		X	Prostate*	X	Bone
	RESPIRATORY	X	Seminal vesicles*		Skeletal muscle
X	Trachea*	X	Ovaries*+		Skin
XX	Lung*	X	Uterus*+	X	All gross lesions and masses*
X	Nose*	X	Vagina		
	Pharynx*				
	Larynx*				

* Recommended for subchronic rodent studies based on Guideline 870.3100
+ Organ weights required for rodent studies
[T] Required only when toxicity or target organ

6. <u>Reproduction toxicity</u> - The caudal, epididymal, and testicular weights, sperm motility, sperm count per gram caudal tissue, and testicular spermatid head count were evaluated at necropsy. Vaginal cytology was evaluated on animals during the two weeks just preceding necropsy, using procedures outlined by Morrissey et al. (1988) (referenced in the report). For the 12 days prior to sacrifice, females were subjected to vaginal lavage with saline. The aspirated cells were air dried onto slides, stained with Toluidine Blue O, and cover slipped. The relative preponderance of leukocytes, nucleated epithelial cells,

and large squamous epithelial cells were used to identify the stages of the estrual cycle.
Sperm motility was evaluated at necropsy as follows: the left epididymis was removed and quickly weighed; the cauda epididymis was removed at the junction of the vas deferens and the corpus epididymis, and then weighed. Warm (37°C) test yolk buffer was applied to two pre-warmed slides, and a small cut was made in the distal cauda epididymis. The sperm that extruded from the epididymis were dispersed throughout the solution, cover-slipped, and counted immediately on a warmed microscope stage. Two independent observers counted the number of moving and non-moving sperm in 5 fields of 30 sperm or less per field. After sperm sampling for motility evaluation, the cauda was placed in phosphate buffered saline (PBS), chopped with a razor blade, and allowed to sit for 15 minutes. The remaining clumps of tissue were removed; the solution was mixed gently, then heat-fixed at 65°C. Sperm density was determined using a hemocytometer. To quantify spermatogenesis, the left testis was weighed, frozen and stored. After thawing, testicular spermatid head count was determined by removing the tunica albuginea and homogenizing the testis in PBS containing 10% DMSO. Homogenization-resistant spermatid nuclei were enumerated using a hemocytometer; the data were expressed as spermatid heads per total testis, and per gram of testis. All above reproduction tissue evaluations were performed in the three high doses and control groups.

II. RESULTS

A. Observations:

1. Clinical signs of toxicity - Diarrhea was noted in the high dose animals for the first 50 days of the treatment, though not thereafter. The incidence and/or severity of this finding were not provided in the report.

2. Mortality - All animals survived until the scheduled necropsy.

B. Body weight and weight gain - A summary of body weight and body weight gain data are provided in table 2. Weekly body weight data was not provided in the report. Body weight and body weight gain were reduced for the top two doses in the males and top dose in females.

Table 2 Body weight (g) and body weight gain (g)[a]

Conc. (ppm)		Initial BW (g)	Final BW (g)	BWG (g)
0	M	115	353	238
	F	95	191	96
3125	M	111	352	241
	F	92	190	98
6250	M	111	338	227
	F	94	194	100
12500	M	113	345	232
	F	96	193	97
25000	M	108	332 (-6%)	224 (-6%)
	F	92	186 (-3%)	94 (-2%)
50000	M	112	290 (-18%)	178 (-25%)
	F	95	181 (-5%)	86 (-10%)

[a] Data was obtained from page 23 of the study report
( ) indicates a percent difference from control

C. Food consumption and compound intake

1. Food consumption - The average food consumption was slightly lower in the high dose groups

compared to control (♂: -12%, ♀: -9%). Weekly food consumption values were not reported.

E. Blood analyses

1. Hematology - Treatment-related hematology findings observed in males were slight increases in hematocrit (2-7%) and RBC (2-7%) at the three high doses, hemoglobin (5-7%) at the two high doses, and platelets (11%) at the high dose. In high dose females, increases in lymphocyte and platelet counts, WBC, MCH, and MCV were noted.

2. Clinical chemistry - Treatment-related dose response increase (♂: 4-22%, ♀: 6-25%; Table B2 pages 50-51 of the study report) in the activity of alkaline phosphatase (ALP) was noted in males and in females across all dose groups. An increase in bile acid concentrations were also noted in the two high dose groups. A treatment-related increase in the alanine aminotransferase activity (ALT) was noted in males starting at 410 mg/kg bw/day. Histopathological findings related to these effects were not reported (histopathology was only conducted for the highest dose and control animals).

G. Sacrifice and pathology -

1. Organ weight - In the two high dose male groups, increases in relative organ weights were observed for liver (~ 15%), right kidney (~ 8-13%), and right testicle (~7-21%). A decrease in the absolute (♂: 7-28%, ♀: 8-12%) and relative (♂:7-13%, ♀: 6-7%) thymus weight was noted across all dose groups. Absolute heart weight (♂:13%, ♀: 6%) was decreased in high dose animals. Relative right kidney weight (~7%) was also increased in high dose females.

2. Gross pathology - Incidences of the findings were not reported; however, it was mentioned that no gross lesions were observed that were considered possibly related to glyphosate administration.

3. Microscopic pathology - Key histopathological findings are reported in Table 3.

Increased incidence and severity of lesions in the parotid and submandibular salivary glands of male and female rats were observed. These lesions were diagnosed as "cytoplasmic alteration" and consisted of basophilic change and hypertrophy of acinar cells. These changes were noted more in the parotid gland in which the normal glandular, eosinophilic staining cytoplasm of the acinar epithelial cells was replaced by basophilic and finely vacuolated cytoplasm. The pathology report indicated that this effect varied in distribution from multifocal in less severe cases, imparting a mottled tinctorial staining appearance to the gland, to diffuse involvement in higher dose animals. In addition, acinar cells appeared swollen, resulting in enlargement of secretory acini and a relative reduction in the number of secretory ducts seen. Nuclei of affected acinar cells were hyperchromatic. In the submandibular salivary gland, similar cytoplasmic tinctorial and hypertrophic effects were observed. No lesions in the sublingual gland were reported.

Incidences of the findings for other tissues and organs were not reported; however, it was mentioned that no lesions were observed that were considered possibly related to glyphosate administration.

Table 3 - Incidence and Severity of Cytoplasmic Alteration of the Parotid and Submandibular Salivary Glands (combined) following the 90 day treatment with glyphosate[a]

Combined Incidence and Severity of Cytoplasmic alteration of the parotid and Submandibular glands						
N	10	10	10	10	10	10
Dose	0	3125	6250	12500	25000	50000
♂	0	6 (1)	10 (1)	10 (2)	10 (3)	10 (3)
♀	0	8 (1)	10 (1)	10 (2)	10 (2)	10 (3)

[a] Data was obtained from page 23 of the study report
( ) Average severity score based on a scale of 1=minimal, 2=mild, 3=moderate, 4= marked

4. Reproductive toxicity - Treatment-related findings included decrease (~10-20%) in sperm counts in the three high doses. Left caudal, epididymal and testicular weights, epididymal sperm motility, total spermatid heads/testes, and total spermatid heads/g caudal tissue were not different from those of controls. High dose females had a longer estrous cycle length (5.4 days vs. 4.9 days) compared to controls.

III. DISCUSSION

A. Investigators' conclusions - "In the 13-week studies, glyphosate did not affect survival of F344/N rats or B6C3F1 mice. Body weight gains were depressed in rats and mice at the 2 highest dose levels; weight gain depression was more severe in males than in females. Kubena et al (1981) reported that body weight gains were reduced (about 50%) in male and female chicks fed a diet containing 6080 ppm of the isopropylamine salt of glyphosate for 21 days, beginning at 1 day of age; the calcium and magnesium content of the tibiotarsus bone was increased compared to controls. There were no differences in body weights in chicks fed a dose of 608 ppm or lower. In the Kubena study (which did not mention feed palatability) and in our 13-week study, the possibility of reduced food intake in the high dose groups cannot be ruled out; more food tends to be spilled when it is not palatable, and our food consumption measurements did not account for scattered feed. Poor palatability of feed containing high concentrations of glyphosate is suggested by the finding that rats drank less water containing Roundup® at 10000 ppm or higher. Another possibility is that the higher concentrations of glyphosate in feed result in poor absorption of dietary components from the GI tract. However, if uncoupling of oxidative phosphorylation, as proposed by Olorunsogo et al. (1979) and Bababunni et al. (1979), is occurring as a result of glyphosate ingestion, then a reduction in weight gain for a given amount of food consumed would be expected.

Hematologic effects in rats dosed with glyphosate were unremarkable and generally consistent with mild dehydration (increases in RBC counts, hematocrit, and hemoglobin concentrations). This conclusion also is supported by the mild increases that occurred at various time points in serum concentrations of urea nitrogen, total protein and albumin. Mild but significant increase in concentrations of TBA and in activities of serum alanine aminotransferase and alkaline phosphatase at multiple time points in male and female rats are consistent with an hepatobiliary effect. These findings likely reflect hepatocellular leakage or perhaps single cell necrosis (ALT) and cholestasis (TBA and ALP). Increases in absolute and relative liver weights in female rats also were suggestive of an effect of glyphosate on the liver, and support the clinical pathology findings. However, the lack of histopathologic evidence for a treatment-related effect on the liver indicates the mild nature of the hepatotoxicity. Vainio et al. (1983) reported an absence of peroxisome proliferation or hypolipidemia in male Wistar rats given Roundup® daily by gavage at 300 mg/kg, 5 times a week for 2 weeks; these daily doses were more than 10-fold lower than those achieved in the highest dose groups in the current study.

Measures of sperm density, or the number/g caudal epididymal tissue, were reduced somewhat in male rats in the 2 highest dose groups; other spermatozoal measurements were not different from controls in rats or mice. There was a slight lengthening of the estrous cycle in high dose female rats, but the biologic significance of these findings, if any, is not known.

It is noteworthy that the U.S. Environmental Protection Agency, after reviewing an unpublished 2-year carcinogenicity study of glyphosate in CD-1 mice, announced that there was "equivocal carcinogenic response, possibly causing a slight increase in the incidence of renal tubular adenomas in male mice at the highest dose tested (30000 ppm)." A carcinogenicity study in rats hast yet to be reviewed (Anonymous,

1991). In the present study, however, the salivary gland was identified as the sole target organ for glyphosate toxicity in both rats and mice. The lesion was diagnosed as cytoplasmic alteration of the acinar epithelial cells, consisting of increased basophilic staining and vacuolation of cytoplasm, and enlargement of cells and acini. This lesion was limited to the parotid gland in mice but affected both parotid and submandibular glands in rats; the sublingual gland was not affected. Salivary gland lesions are relatively uncommon in toxicity studies; however, both spontaneous and chemically-induced changes of a similar nature to those seen in the glyphosate study have been described. So called "basophilic hypertrophic foci" occasionally may be seen as a spontaneous lesion in the parotid gland or rats and mice (Chiu and Chen, 1986); however, these are infrequent and focal in nature. More extensive and diffuse basophilic and hypertrophic change has been described in subchronic studies with some chemicals, such as doxylamine (Jackson and Blackwell, 1988) and methapyrilene (Jackson and Sheldon, 1984). By far, the most extensive and detailed studies of these changes in salivary glands have been done with sympathomimetic agents -- for example, the adrenergic agonist, isoproterenol, which induces striking morphologic changes in salivary glands (Schneyer, 1962; Fukuda, 1968). As with glyphosate's effects on the salivary glands, isoproterenol affects the parotid and submandibular glands but not the sublingual. This is due to the fact that, in the rat, the acini of the parotid and submandibular are richly supplied with adrenergic fibers, while the sublingual gland is devoid of adrenergic innervation (Nordenfelt, 1967). Because glyphosate and isoproterenol are similar in both morphologic effects induced in the salivary glands and the gland specificity of those effects, it was hypothesized that glyphosate-related lesions were mediated through an adrenergic mechanism. A study was designed to test this hypothesis."

B. <u>Reviewer comments</u> - The study author's conclusions are acceptable regarding the target organ. Some findings were also considered treatment related because of statistical significance. The study reviewer evaluated the magnitude of change, dose response, and other contextual information available to consider findings treatment related.

All animals survived to necropsy. Diarrhea was noted in the high dose animals. This effect was possibly related to consumption of glyphosate at high concentrations in the diet which was also reflected in lower average food consumption in the high dose animals compared to controls. Body weight and body weight gain were reduced in the top two doses in males and top dose in females.

Increases in the activities of ALP in the treated animals were noted. In addition, increased relative organ weights were noted in males. These findings are consistent of a hepatobiliary effect that can be attributed to glyphosate administration.

A decrease in absolute and relative thymus weight was noted across all dose groups. Absolute heart weight was increased in the high dose animals. Relative right kidney and right testis weights were increased in top two male groups. In high dose females, relative right kidney weight was increased. The histopathology findings for these organs were not reported (these tissues were examined for high dose and control groups). The study author stated that no treatment-related lesions from the microscopic examinations besides the salivary glands toxicity.

A dose-related increase in the incidence and severity of cytoplasmic alteration of the parotid and submandibular salivary glands was noted in the microscopic evaluations. The pathology report indicated that this effect varied in distribution from multifocal in less severe cases, imparting a mottled tinctorial staining appearance to the gland, to diffuse involvement in higher dose animals. In addition, acinar cells appeared swollen, resulting in enlargement of secretory acini and a relative reduction in the number of secretory ducts seen. Nuclei of affected acinar cells were hyperchromatic.

A decrease in sperm counts was noted at three high doses. Sperm measurements were not performed in the two low dose groups. Longer estrous cycle was noted in the high dose group. No other treatment

related finding was noted in the evaluations of reproductive tissues.

PMRA: The LOAEL is 205/213 mg/kg bw/day for males and females based on dose related increase in the incidence and severity of cytoplasmic alterations in the parotid and submandibular salivary glands of male and female rats. A NOAEL was not determined.

EPA: The NOAEL is 410 and 421 mg/kg/day for males and females, respectively. The LOAEL is 811/844 mg/kg/day based on mild cytoplasmic alterations in the parotid and submandibular salivary glands. The alterations at lower doses were scored as minimal in severity and not considered adverse.

This study is considered acceptable and classified as appropriate for quantitative use for hazard characterization of glyphosate. It is recognized that raw data was not provided in the study report; however, this report provided a relatively comprehensive understanding of the conditions under which the study was conducted and of the data generated by the study. In addition, the effects noted in this study (e.g. salivary glands toxicity) are aligned with other lines of evidence throughout the glyphosate toxicology database (e.g. other short and long term studies have reported salivary gland toxicity).

C. Study deficiencies -
The following is a list of deficiencies according to OPPTS 870.3100; OECD 408 test guidelines.

1. Relative humidity levels should be between 30-70% compared to 40%-89%
2. The actual concentration, stability and homogeneity of the test substance in the diet were not reported or determined.
3. Ophthalmological examination was not conducted.
4. A measure of blood clotting time/potential was not examined.
5. Electrolyte levels, glucose, total cholesterol were not examined in clinical chemistry assessment.
6. Spleen, brain, ovaries, epididymides, uterus were not weighed.
7. Weekly body weight and food consumption data, gross pathology data, and histopathology data on other organs besides salivary glands were not reported.
8. Raw data was not included in the study report.

These deficiencies are not expected to impact the regulatory outcome of this study.

Study Type: Short-Term Oral (90-day) (rodent) [feeding] - Mice; OPPTS 870.3100 (rodent); OECD 408.

Test Material (purity): Glyphosate (99% a.i.)

Synonyms: Glyphosate, technical grade; Glycine, N-(phosphonomethyl); N-phosphono-methyl glycine; N-(phosphonomethyl)glycine; MON0573.

Citation: NTP Technical Report on Toxicity Studies of Glyphosate Administered in Dosed Feed to F344/N Rats and B6C3F1 Mice. Laboratory name: Southern Research Institute, Birmingham, AL. Laboratory report number: Glyphosate, NTP Toxicity Report Number 16. Study report date: July 1992.

Sponsor: N/A

MRID: N/A

Executive Summary: In a short term chronic toxicity study, glyphosate (99%) was administered to 10 B6C3F1mice/sex/group at dietary concentrations of 0, 3125, 6250, 12500, 25000, or 50000 ppm (equivalent to 0, 507, 1065, 2273, 4776, or 10780 mg/kg bw/day for males and 753, 1411, 2707, 5846, 11977 mg/kg bw/day for females, respectively) for 90 days. Evaluation of the reproductive tract tissues and estrual cycle length were also incorporated in the design of the study and examined at the three high doses and control groups.

Two female animals (one from the control group and one from the high dose group) were sacrificed before the end of the treatment. Necropsy findings on these animals were not provided to confirm or dismiss a treatment-related cause of death. No clinical signs of toxicity were reported. A dose related decrease in the body weight and body weight gain of the animals were noted starting at the third high dose group. Average daily food consumption was comparable across the treated and control groups. Hematology and clinical chemistry examinations were not conducted.

A non-dose related increase in the absolute and relative weight of heart was observed starting at the second low dose group in males. A non-dose related increase in the absolute and relative weights of right kidney and lungs was observed starting at low dose males. Increases in the relative liver weight and relative right testis weight at the two high dose male groups were also noted. In females, a dose related decrease in the absolute heart weight was observed at three high dose groups. A dose related decrease in the absolute liver weight was noted at the two high female dose groups. A non-dose related decrease in the absolute and relative thymus weight was noted in the three high dose female groups. Histopathology examination of control and high dose animals did not reveal related findings. A treatment-related effect was not observed in the examination of the reproductive tract tissues and estrual cycle length.

Dose related increase in the incidence and severity of lesions in the parotid salivary glands of male and female mice were observed. These lesions were diagnosed as “cytoplasmic alteration” and consisted of basophilia of the acinar cells. In more severely affected glands, the cells and acini also appeared enlarged with an associated relative reduction in the number of ducts. PMRA believes this will likely result in altered general function of the salivary glands organ; therefore, this finding was considered adverse.

PMRA: The NOAEL is 507/753 mg/kg bw/day for males/females. The LOAEL is 1065/1411 mg/kg bw/day for males/females based on dose related increase in the incidence and severity of

cytoplasmic alterations in the parotid salivary glands.

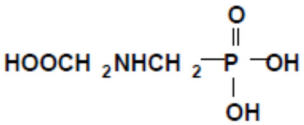
EPA: The NOAEL is 1065 and 1411 mg/kg/day for males and females, respectively. The LOAEL is 2273/2707 mg/kg/day based on cytoplasmic alterations (average severity scores = mild in males, greater than minimal but less than mild in females) in the parotid and submandibular salivary glands. The alterations at lower doses were scored as minimal in severity and not considered adverse.

Although this study was not conducted according the guideline requirement for a subchronic oral study (OPPTS 870.3100; OECD 408), it is considered acceptable and classified as appropriate for quantitative use for hazard characterization of glyphosate.

Compliance: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were not provided. However, this study was peer-reviewed by NTP internally. The peer-review panel determined that the design and conditions of this study was appropriate and ensured that the toxicity study report presented the experimental results and conclusions thoroughly and clearly.

I. MATERIALS AND METHODS

A. Materials

Test material:	Glyphosate
Description:	White solid, stable for at least 3 weeks in room temperature in the dark
Lot/Batch #:	Obtained from Monsanto Agricultural Products (St. Louis)
Purity:	99 % a.i.
CAS #:	1071-83-6
Structure:	

Test species:	Mice
Strain:	B6C3F1
Age at start:	49 days of age
Weight at start:	♂: 23-23.5g ♀:18.2-18.9g
Source:	Simonsen Laboratories (Gilroy, CA, USA)
Housing:	1 per cage
Diet:	NIH-07 feed was provided *ad libitum*
Water:	NIH-07 water was provided *ad libitum*
Environmental conditions:	**Temperature:** 67-74°F **Humidity:** 40-89% **Air changes:** 10/hr **Photoperiod:** 12 hours dark/12 hours light
Acclimation:	11 days

B. Study design and methods

1. Study experimentation dates - Start: May -1988 End: September - 1988

2. Animal assignment - Animals were assigned randomly (stratified weight method first and then assigned to study groups in a random order) to the test groups noted in Table 1. Copies of proprietary reports of toxicity studies performed by Monsanto Corporation were made available to the NTP for use in designing its glyphosate studies.

Table 1: Study Design[a]

Dose (mg/kg bw/d)		Conc. in Diet (ppm)	Number of Animals Main Study - 3 months	
Male	**Female**		**Male**	**Female**
0	**0**	0	10	10
507	753	3125	10	10
1065	1411	6250	10	10
2273	2707	12500	10	10
4776	5846	25000	10	10
10780	11977	50000	10	10

[a] Data were extracted from page 12 of the study report

3. Diet preparation and analysis - The report stated that glyphosate in diet was stable for at least 3

weeks at room temperature when stored in the dark. Further details regarding the concentration, stability, and homogeneity analysis of the test compound in the diet were not provided.

4. Statistics -

"Two approaches were employed to assess the significance of pairwise comparisons between dosed and control groups in the analysis of continuous variables. Organ and body weight data, which are approximately normally distributed, were analyzed using the parametric multiple comparisons procedures of Williams (1971, 1972, 1986) and Dunnett (1955). Clinical pathology and hematology data, which typically have skewed distributions were analyzed using nonparametric multiple comparisons methods of Shirley (1977) and Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of dose-response trends and to determine whether a trend-sensitive test (Williams, Shirely) was more appropriate for pairwise comparisons than a test capable of detecting departures from monotonic dose response (Dunnett, Dunn). If the P-value from Jonchkheere's test was greater than or equal to 0.10, Dunn's or Dunnett's test was used rather than Shirley's or Williams's test. The outlier test of Dixon and Massey (1951) was employed to detect extreme values. No value selected by the outlier test was eliminated unless it was at least twice the next largest value or at most half of the next smallest value.

Analysis of Vaginal Cytology Data

Since the data are proportions (the proportion of the observation period that an animal was in a given estrous state), an arcsine transformation was used to bring the data into closer conformance with normality assumptions. Treatment effects were investigated by applying a multivariate analysis of variance (Morrison, 1976) to the transformed data to test for the simultaneous equality of measurements across dose levels."

C. Methods

1. Observations - Animals were inspected twice daily for mortality/moribundity, and once a week for clinical signs of toxicity.

2. Body weight - Animals were weighed weekly.

3. Food consumption and compound intake - Food consumption for each animal was determined as average food consumption in g/animal/day. Compound intake (mg/kg bw/day) values were estimated as time-weighted averages from the consumption and body weight gain data.

4. Hematology and clinical chemistry – Hematology and clinical chemistry measurements were not conducted.

5. Sacrifice and pathology - All animals were sacrificed with carbon dioxide at the end of the study and were subjected to gross pathological examination. The checked (X) tissues were collected for histological examination from the control and high dose group animals. The salivary gland was examined in all dose groups. In addition, the (XX) organs were weighed. A necropsy was performed on all animals. Tissues were preserved in 10% neutral buffered formalin. Following dehydration and embedding, tissues were sectioned at approximately 5µM, stained with hematoxylin and eosin (H&E), and then examined microscopically. Blood smears were prepared from animals for genetic toxicology studies (determination of micronuclei in erythrocytes). After completion of the histopathological evaluation by laboratory pathologist on site, the slides, paraffin blocks, and residual wet tissues were sent to the NTP Archives for inventory, slide/block match, and wet tissue audit. The slides, individual animal data records, and pathology tables were sent to independent pathology laboratory where quality assessment was performed.

The results were reviewed and evaluated through an NTP Pathology Review. The reported data represented a consensus of contractor and review pathologists.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
	Tongue		Aorta*	X	Brain*+
X	Salivary glands*	XX	Heart*+		Peripheral nerve*
X	Esophagus*		Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	X	Pituitary*
X	Duodenum*	X	Spleen*+		Eyes (optic nerve)*
X	Jejunum*	XX	Thymus*+		**GLANDULAR**
X	Ileum*			X	Adrenal gland*+
X	Cecum*		**UROGENITAL**		Lacrimal gland[T]
X	Colon*	XX	Kidneys*+	X	Mammary gland*
X	Rectum*	X	Urinary bladder*	X	Parathyroid*
XX	Liver*+	XX	Testes*+	X	Thyroid*
X	Pancreas*	X	Epididymides*+		**OTHER**
		X	Prostate*	X	Bone
	RESPIRATORY	X	Seminal vesicles*		Skeletal muscle
X	Trachea*	X	Ovaries*+		Skin
XX	Lung*	X	Uterus*+	X	All gross lesions and masses*
X	Nose*	X	Vagina		
	Pharynx*				
	Larynx*				

* Recommended for subchronic rodent studies based on Guideline 870.3100
\+ Organ weights required for rodent studies
[T] Required only when toxicity or target organ

6. <u>Reproduction toxicity</u> - The reproductive tract tissue evaluations were performed in the three high dose and control groups. The caudal, epididymal, and testicular weights, sperm motility, sperm count per gram caudal tissue, and testicular spermatid head count were evaluated at necropsy. Vaginal cytology was evaluated in animals during the two weeks just preceding necropsy, using procedures outlined by Morrissey et al. (1988) (referenced in the report). For the 12 days prior to sacrifice, females were subjected to vaginal lavage with saline. The aspirated cells were air dried onto slides, stained with Toluidine Blue O, and cover slipped. The relative preponderance of leukocytes, nucleated epithelial cells, and large squamous epithelial cells were used to identify the stages of the estrual cycle.
Sperm motility was evaluated at necropsy as follows: the left epididymis was removed and quickly weighed; the cauda epididymis was removed at the junction of the vas deferens and the corpus epididymis, and then weighed. Warm (37°C) Tyrodes buffer was applied to two pre-warmed slides, and a small cut was made in the distal cauda epididymis. The extruded sperm from the epididymis were dispersed throughout the solution, cover-slipped, and counted immediately on a warmed microscope stage. Two independent observers counted the number of moving and non-moving sperm in 5 fields of 30 sperm or less per field. After sperm sampling for motility evaluation, the cauda was placed in phosphate buffered saline (PBS), chopped with a razor blade, and allowed to sit for 15 minutes. The remaining clumps of tissue were removed; the solution was mixed gently, then heat-fixed at 65°C. Sperm density was determined using a hemocytometer. To quantify spermatogenesis, the left testis was weighed, frozen and stored. After thawing, testicular spermatid head count was determined by removing the tunica albuginea and homogenizing the testis in PBS containing 10% DMSO. Homogenization-resistant spermatid nuclei were enumerated using a hemocytometer; the data were expressed as spermatid heads per total testis, and per gram of testis.

II. RESULTS

A. <u>Observations</u>:

1. Clinical signs of toxicity - Clinical signs of toxicity were not reported.

2. Mortality - There were two early (unscheduled) deaths in the study. The study author stated that a control female was accidentally killed, and a high dose female died from undetermined causes. Necropsy data on these animals (especially the treated animal) were not reported to either confirm or dismiss a treatment-related cause.

B. Body weight and weight gain - A summary of body weight and body weight gain data are provided in table 2. A dose related decrease in the overall body weight and body weight gain was noted in the three high dose groups. Weekly body weight measurements were not reported.

Table 2 Body weight (g) and body weight gain (g)[a]

Conc. (ppm)		Initial BW (g)	Final BW (g)	BWG (g)
0	M	23.5	32.1	8.6
	F	18.9	27.9	9.0
3125	M	23.2	31.1	7.9
	F	18.4	28.6	10.2
6250	M	23.4	31.5	8.1
	F	18.2	26.2	8.0
12500	M	23.2	30.3 (-6%)	7.1 (-17%)
	F	18.8	26.9 (-4%)	8.1 (-10%)
25000	M	23.0	28.6 (-11%)	5.6 (-35%)
	F	18.5	26.2 (-6%)	7.7 (-14%)
50000	M	23.5	26.7 (-17%)	3.2 (-63%)
	F	18.5	25.1 (-10%)	6.6 (-27%)

[a] Data was obtained from page 23 of the study report
( ) indicates a percent difference from control

C. Food consumption and compound intake

1. Food consumption - The average daily food consumption was comparable across all treated and control groups. Weekly food consumption values were not reported.

E. Blood analyses

1. Hematology – Hematology analyses were not conducted in this study. However, blood smears were analyzed to determine micronuclei in the erythrocytes for genotoxic effects of glyphosate (if any). The results of this study is captured in another review

2. Clinical chemistry - Clinical chemistry measurements were not conducted.

G. Sacrifice and pathology -

1. Organ weight - Organ weight data are summarized in table 3. Individual organ weight data was not provided in this study.

A non-dose related increase in the absolute and relative heart weight (abs.: 6-16%, rel.: 10-23%) was observed starting at the second low dose group in males. A non-dose related increase in the absolute and relative weights of right kidney (abs.: 6-15%, rel.: 7-23%) and lungs (abs.: 9-18%, rel.: 9-28%) was also observed starting at low dose males. Increase in the relative liver weight (~ 8%) and relative right testis weight (~ 10-18%) at the two high dose male groups was also noted. The magnitude of the increase in

these findings was dose related in the first three or four doses and plateaued in the following higher dose(s). An initial screening of the glyphosate animal metabolism data from various studies revealed that the unchanged parent compound (glyphosate) was excreted in the feces suggesting poor systemic absorption from the oral route. Metabolism studies that were conducted at dose levels exceeding the limit dose (i.e. at the dose levels used in this study) were not available. Metabolism data should be further examined to relate the plateauing trend in the organ weight data with absorption data (e.g. if the plateauing trend is likely due to saturation of absorption or poor systemic absorption of glyphosate from the oral route). Such findings was also likely due to poor selection of four dose levels that were well in excess of the limit dose (= 1000 mg/kg bw/day) of testing.

In females, a dose related decrease in the absolute heart weight (~ 6-14%) was observed at the three high dose groups. A dose related decrease in the absolute liver weight (~ 8-14%) was noted at the two high female dose groups. A non-dose related decrease in the absolute and relative thymus weight (abs.: 14-21%, rel.: 10-18%) was noted in the three high dose female groups. The organ weight data showed contradictory results in males and females (i.e. increase in the heart weight in males and decrease in the heart weight in females). An initial screen of limited metabolism data did not clearly dismiss or confirm gender-specific differences in the absorption that could relate to this contradictory finding; however, this conclusion can change upon further examination of metabolism data (if available).

The study author stated that no histopathological lesions were noted in these organs; however, only the control and high dose animals were examined. The coefficient of variations (standard deviation/mean) for the organ data were considered acceptable by the reviewer as they were below 20%. Historical control data were not provided to examine whether the concurrent control values were aberrant. In addition, clinical chemistry and hematology data were not part of the design of this study to provide further relating evidence. Therefore, in the absence of these data, the above-mentioned changes in the organ weights were considered treatment related. In addition, sporadic, but treatment-related findings have been noted in other toxicology studies in these organs (especially in kidneys and lungs) to suggest that these organs can be a target of glyphosate toxicity.

Table 3. Organ weights Data[a]

♂	**0**	**3125 ppm**	**6250 ppm**	**12500 ppm**	**25000 ppm**	**50000 ppm**
Abs. heart	0.145 ± 0.003	0.149 ± 0.003	0.161 ± 0.006	0.168 ± 0.006*	0.153 ± 0.007	0.153 ± 0.007
Rel. heart	4.56 ± 0.14	4.71 ± 0.11	4.98 ± 0.17	5.31 ± 0.22**	5.21 ± 0.20**	5.60 ± 0.20**
Abs. kidney	0.279 ± 0.006	0.295 ± 0.006	0.313 ± 0.011	0.320 ± 0.009*	0.316 ± 0.014	0.278 ± 0.012
Rel. kidney	8.74 ± 0.15	9.35 ± 0.24	9.68 ± 0.31*	10.07 ± 0.27**	10.75 ± 0.40**	10.18 ± 0.31**
Abs. liver	1.39 ± 0.05	1.46 ± 0.07	1.54 ± 0.06	1.43 ± 0.05	1.38 ± 0.04	1.28 ± 0.04
Rel. liver	43.4 ± 0.9	45.8 ± 0.8	47.5 ± 1.3*	45.0 ± 0.9*	47.0 ±0.8*	47.1 ± 1.0*
Abs. lungs	0.159 ± 0.003	0.173 ± 0.007	0.188 ± 0.012	0.183 ± 0.005	0.179 ± 0.010	0.174 ± 0.007
Rel. lungs	5.00 ± 0.16	5.45 ± 0.16	5.81 ± 0.37*	5.78 ± 0.20*	6.11 ± 0.35**	6.38 ± 0.20**
Abs. testis	0.118 ± 0.002	0.117 ± 0.003	0.122 ± 0.003	0.116 ± 0.003	0.120 ± 0.003	0.119 ± 0.004
Rel. testis	3.71 ± 0.12	3.69 ± 0.10	3.77 ± 0.07	3.66 ± 0.06	4.08 ± 0.10**	4.37 ± 0.11**
abs. thymus	0.036 ± 0.002	0.037 ± 0.002	0.042 ± 0.002	0.040 ± 0.002	0.036 ± 0.002	0.038 ± 0.002
Rel. thymus	1.14 ± 0.08	1.15 ± 0.05	1.31 ± 0.07	1.26 ± 0.05	1.21 ± 0.05	1.39 ± 0.05**
♀	**0**	**3125 ppm**	**6250 ppm**	**12500 ppm**	**25000 ppm**	**50000 ppm**
Abs. heart	0.143 ± 0.008	0.138 ± 0.004	0.140 ± 0.007	0.135 ± 0.004	0.132 ± 0.005	0.124 ± 0.004*
Rel. heart	4.98 ± 0.21	4.83 ± 0.16	5.17 ± 0.26	4.72 ± 0.17	4.90 ± 0.20	4.86 ± 0.18
Abs. kidney	0.214 ±0.009	0.235 ± 0.007	0.217 ± 0.009	0.222 ± 0.005	0.212 ± 0.005	0.212 ± 0.006
Rel. kidney	7.45 ± 0.21	8.22 ± 0.28	8.02 ± 0.31	7.75 ± 0.18	7.87 ± 0.23	8.28 ± 0.22
Abs. liver	1.37 ± 0.06	1.37 ± 0.03	1.33 ± 0.04	1.32 ± 0.03	1.27 ± 0.03	1.18 ± 0.03**
Rel. liver	47.5 ± 1.3	47.8 ± 1.1	49.1 ±0.9	45.9 ±1.0	46.9 ±0.7	46.1 ± 0.9
Abs. lungs	0.182 ±0.007	0.175 ±0.005	0.181 ± 0.011	0.180 ± 0.005	0.167 ± 0.007	0.171 ± 0.006
Rel. lungs	6.33 ± 0.19	6.12 ± 0.22	6.69 ± 0.39	6.29 ± 0.21	6.21 ± 0.31	6.67 ± 0.22
abs. thymus	0.056 ± 0.003	0.049 ± 0.002	0.055 ± 0.004	0.048 ± 0.003	0.044 ± 0.003**	0.045± 0.002**
Rel. thymus	1.94 ±0.08	1.71 ±0.06	2.01 ±0.15	1.68 ±0.11	1.61 ±0.09	1.75 ±0.07

[a] Data was obtained from page 44 of the study report
Organ weights and body weights are given in grams, relative organ weights (organ-weight-to-body-weight ratios are given as mg organ weight/g body weight [mean ± standard error]) are calculated by the study author.
* Statistically significantly different (P 0.05) from the control group by Williams' test or Dunnett's test
* Statistically significantly different (P 0.01) from control group by Williams' test or Dunnett's test

2. <u>Gross pathology</u> - A "dark" salivary gland in a high dose male was noted at gross necropsy examinations. This finding is consistent with the treatment-related histopathological findings in the salivary glands. Incidences of the findings were not reported; however, it was mentioned that no gross lesions were observed that were considered possibly related to glyphosate administration.

3. <u>Microscopic pathology</u> - Key histopathological findings are reported in Table 3.

Dose related increase in the incidence and severity of lesions in the parotid salivary glands of male and female mice were observed. These lesions were diagnosed as "cytoplasmic alteration" and consisted of basophilia of the acinar cells. In more severely affected glands, the cells and acini also appeared enlarged with an associated relative reduction in the number of ducts. Similar treatment-related effects were not noted on submandibular and sublingual salivary glands. This finding was considered treatment-related.

Incidences of the findings for other tissues and organs were not reported; however, it was mentioned that no lesions were observed that were considered possibly related to glyphosate administration.

Table 3 - Incidence and Severity of Cytoplasmic Alteration of the Parotid Salivary Glands (combined) following the 90 day treatment with glyphosate[a]

Combined Incidence and Severity of Cytoplasmic alteration of the parotid glands						
N	10	10	10	10	10	10
Dose (ppm)	0	3125	6250	12500	25000	50000
♂	0	0	5 (1)	9 (1.6)	10 (2.8)	10 (4)
♀	0	0	2 (1)	9 (1.3)	10 (2.1)	10 (3.1)

[a] Data was obtained from page 23 of the study report
( ) Average severity score based on a scale of 1=minimal, 2=mild, 3=moderate, 4= marked

4. <u>Reproductive toxicity</u> – No treatment-related effects were observed in the evaluations of the reproductive tissues and estrual cycle length.

III. DISCUSSION

A. <u>Investigators' conclusions</u> - "In the 13-week studies, glyphosate did not affect survival of F344/N rats or B6C3F1 mice. Body weight gains were depressed in rats and mice at the 2 highest dose levels; weight gain depression was more severe in males than in females. Kubena et al (1981) reported that body weight gains were reduced (about 50%) in male and female chicks fed a diet containing 6080 ppm of the isopropylamine salt of glyphosate for 21 days, beginning at 1 day of age; the calcium and magnesium content of the tibiotarsus bone was increased compared to controls. There were no differences in body weights in chicks fed a dose of 608 ppm or lower. In the Kubena study (which did not mention feed palatability) and in our 13-week study, the possibility of reduced food intake in the high dose groups cannot be ruled out; more food tends to be spilled when it is not palatable, and our food consumption measurements did not account for scattered feed. Poor palatability of feed containing high concentrations of glyphosate is suggested by the finding that rats drank less water containing Roundup® at 10000 ppm or higher. Another possibility is that the higher concentrations of glyphosate in feed result in poor absorption of dietary components from the GI tract. However, if uncoupling of oxidative phosphorylation, as proposed by Olorunsogo et al. (1979) and Bababunni et al. (1979), is occurring as a result of glyphosate ingestion, then a reduction in weight gain for a given amount of food consumed would be

expected.

It is noteworthy that the U.S. Environmental Protection Agency, after reviewing an unpublished 2-year carcinogenicity study of glyphosate in CD-1 mice, announced that there was "equivocal carcinogenic response, possibly causing a slight increase in the incidence of renal tubular adenomas in male mice at the highest dose tested (30000 ppm)." A carcinogenicity study in rats has yet to be reviewed (Anonymous, 1991). In the present study, however, the salivary gland was identified as the sole target organ for glyphosate toxicity in both rats and mice. The lesion was diagnosed as cytoplasmic alteration of the acinar epithelial cells, consisting of increased basophilic staining and vacuolation of cytoplasm, and enlargement of cells and acini. This lesion was limited to the parotid gland in mice but affected both parotid and submandibular glands in rats; the sublingual gland was not affected. Salivary gland lesions are relatively uncommon in toxicity studies; however, both spontaneous and chemically-induced changes of a similar nature to those seen in the glyphosate study have been described. So called "basophilic hypertrophic foci" occasionally may be seen as a spontaneous lesion in the parotid gland or rats and mice (Chiu and Chen, 1986); however, these are infrequent and focal in nature. More extensive and diffuse basophilic and hypertrophic change has been described in subchronic studies with some chemicals, such as doxylamine (Jackson and Blackwell, 1988) and methapyrilene (Jackson and Sheldon, 1984). By far, the most extensive and detailed studies of these changes in salivary glands have been done with sympathomimetic agents -- for example, the adrenergic agonist, isoproterenol, which induces striking morphologic changes in salivary glands (Schneyer, 1962; Fukuda, 1968). As with glyphosate's effects on the salivary glands, isoproterenol affects the parotid and submandibular glands but not the sublingual. This is due to the fact that, in the rat, the acini of the parotid and submandibular are richly supplied with adrenergic fibers, while the sublingual gland is devoid of adrenergic innervation (Nordenfelt, 1967). Because glyphosate and isoproterenol are similar in both morphologic effects induced in the salivary glands and the gland specificity of those effects, it was hypothesized that glyphosate-related lesions were mediated through an adrenergic mechanism. A study was designed to test this hypothesis."

B. <u>Reviewer comments</u> - The study author's conclusions are acceptable regarding the target organ. Some findings (e.g. changes in the organ weight data) were also considered treatment related because of statistical significance. The study reviewer evaluated the magnitude of change, dose response, and other contextual information available to consider findings treatment related and/or adverse.

Two female animals (one from the control group and one from the high dose group) were sacrificed before the end of the treatment. Necropsy findings on these animals were not given to confirm or dismiss a treatment-related cause of death. No clinical signs of toxicity were reported. A dose related decrease in the body weight and body weight gain of the animals were noted starting at the third high dose group. Hematology and clinical chemistry examinations were not conducted.

A non-dose related increase in the absolute and relative weight of heart was observed starting at the second low dose group in males. A non-dose related increase in the absolute and relative weights of right kidney and lungs was observed starting at low dose males. Increases in the relative liver weight and relative right testis weight at the two high dose male groups were also noted. In females, a dose related decrease in the absolute heart weight was observed in the three highest dose groups. A dose related decrease in the absolute liver weight was noted at the two high female dose groups. A non-dose related decrease in the absolute and relative thymus weight was noted in the three high dose female groups. The study reviewer considered these changes in the organ weight data as treatment related.

Dose related increase in the incidence and severity of lesions in the parotid salivary glands of male and female mice were observed. These lesions were diagnosed as "cytoplasmic alteration" and consisted of basophilia of the acinar cells. In more severely affected glands, the cells and acini also appeared enlarged with an associated relative reduction in the number of ducts. PMRA believes this will likely result in

altered general function of the salivary glands; therefore, this finding was considered adverse.

PMRA: The NOAEL is 507/753 mg/kg bw/day for males/females. The LOAEL is 1065/1411 mg/kg bw/day for males/females based on dose related increase in the incidence and severity of cytoplasmic alterations in the parotid salivary glands.

EPA: The NOAEL is 1065 and 1411 mg/kg/day for males and females, respectively. The LOAEL is 2273/2707 mg/kg/day based on cytoplasmic alterations (average severity scores = mild in males, greater than minimal but less than mild in females) in the parotid and submandibular salivary glands. The alterations at lower doses were scored as minimal in severity and not considered adverse.

This study is considered acceptable and classified as appropriate for quantitative use for hazard characterization of glyphosate. It is recognized that raw data was not provided in the study report; however, this report provided a relatively comprehensive understanding of the conditions under which the study was conducted and of the data generated by the study. In addition, the effects noted in this study (e.g. salivary glands toxicity) are aligned with other lines of evidence throughout the glyphosate toxicology database (e.g. other short and long term studies have reported salivary gland toxicity).

C. Study deficiencies -
The following is a list of deficiencies according to OPPTS 870.3100; OECD 408 test guidelines.

1. Relative humidity levels should be between 30-70% compared to 40%-89%
2. The actual concentration, stability and homogeneity of the test substance in the diet were not reported or determined.
3. An additional satellite group of ten animals (five per sex) in the control and high dose group should have been included for observation, after treatment period, for reversibility or persistence of any toxic effects; in this case, mostly salivary gland toxicity.
4. Sensory reactivity to stimuli of different types (e.g. auditory, visual, and proprioceptive stimuli), assessment of grip strength and motor activity assessment were not conducted.
5. Spleen, brain, ovaries, epididymides, uterus were not weighed.
6. Weekly body weight and food consumption data, gross pathology data, and histopathology data on other organs besides salivary glands were not reported.
7. Raw data was not included in the study report.
8. Four dose levels were well in excess of the limit dose of testing (1000 mg/kg bw/day). The dose selection and design of this study should have included more dose levels lower than 1000 mg/kg bw/day compared to using multiple excessive doses. From the body weight data, maximum tolerated dose was reached at 25000 ppm. Reaching MTD is not considered a required result of a short term study.

These deficiencies are not expected to impact the regulatory outcome of this study.

Study Type: Mechanistic Study (rodent) [feeding] - Rat; OPPTS 870.3100 (rodent); OECD 408.

Test Material (purity): Glyphosate (99% a.i.)

Synonyms: Glyphosate, technical grade; Glycine, N-(phosphonomethyl); N-phosphono-methyl glycine; N-(phosphonomethyl)glycine; MON0573; MON 2139.

Citation: NTP Technical Report on Toxicity Studies of Glyphosate Administered in Dosed Feed to F344/N Rats and B6C3F1 Mice. Laboratory name: Southern Research Institute, Birmingham, AL. Laboratory report number: Glyphosate, NTP Toxicity Report Number 16. Study report date: July 1992.

Sponsor: N/A

MRID: N/A

Executive Summary: In a 14-day study conducted because of the morphologic similarity between salivary gland changes noted in the 13-week studies of glyphosate and salivary gland lesions reported to result from the treatment of a β-adrenergic receptor agnonist (i.e. isoproterenol), five groups of four male F344 rats were used in the following manner. Group 1 was fed control diet and was administered the vehicle by continuous subcutaneous infusion by osmotic minipumps. Group 2 and 3 were fed glyphosate @ 50000 ppm in diet and were concurrently administered the vehicle or propranolol (a β-adrenergic receptor antagonist) @ 1.2 mg/kg bw/day by osmotic minipumps respectively. Group 4 was fed control diet and was concurrently administered isoproterenol (a β-adrenergic receptor agonist) @ 1.0 mg/kg bw/day by the minipumps. Group 5 was fed control diet and was concurrently administered isoproterenol and propranolol by the minipumps.

Softer and wetter feces were noted in the glyphosate fed groups by day 7 of the study during the observation of clinical signs of toxicity. Decreased body weight gain was also observed in the glyphosate fed animals. Parotid and submandibular glands weights were increased in the glyphosate fed groups more than other groups. Increased incidence of hypertrophy, cytoplasmic alteration, enlargement of acini and a reduction in the number of ducts were observed in the parotid (groups 2 through 5) and submandibular glands (groups 2 and 3). This effect was more pronounced in group 2 in which animals were fed glyphosate and administered the vehicle concurrently via the pumps. Propranolol administered concurrently with glyphosate suppressed the severity of this effect in parotid gland but did not have detectable inhibitory effect in the submandibular gland. Administration of isoproterenol alone or with propranolol (groups 4 and 5) resulted in similar effects in the parotid glands but no detectable effects in the submandibular glands.

This study by itself did not provide adequate evidence to show that glyphosate has agonistic activity in the β-adrenergic receptors. The salivary glands effects observed in this study (or other studies with glyphosate) are not the only effect that would be observed from the agonistic activity of a compound at β-receptors. Other organs and tissues that are also innervated with these receptors should have been examined to show whether glyphosate acts as an agonist of the β- receptors.

The thickened secretions from the salivary glands were perhaps shown in this study indirectly by the electron micrographs of the salivary glands that indicated an increase in size and number of secretory granules within the acinus cells. The examination of these endpoints are generally not required in the

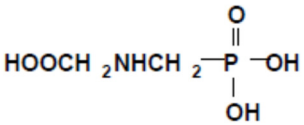
toxicity studies (except glucose levels = increased blood sugar, and dilated pupil of eyes in the clinical observation of these studies).

Overall, this study is considered acceptable and classified as appropriate for qualitative use for hazard characterization of glyphosate. This study was conducted because of the morphologic similarity between a salivary gland change noted in the 13-week studies of glyphosate and a salivary gland lesion previously reported to result from treatment with the adrenergic agnonist, isoproterenol.

Compliance: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were not provided. However, this study was peer-reviewed by NTP internally. The peer-review panel determined that the design and conditions of this study was appropriate and ensured that the toxicity study report presented the experimental results and conclusions thoroughly and clearly.

I. MATERIALS AND METHODS

A. Materials

Test material:	Glyphosate
Description:	White solid, stable for at least 3 weeks in room temperature in the dark
Lot/Batch #:	Obtained from Monsanto Agricultural Products (St. Louis)
Purity:	99 % a.i.
CAS #:	1071-83-6
Structure:	

Test species:	Male Rats
Strain:	F344/N
Age at start:	Not stated
Weight at start:	♂: 200-250 □
Source:	Charles River Laboratories (Raleigh, NC)
Housing:	Not stated
Diet:	NIH-07 feed was provided *ad libitum*
Water:	Not stated
Environmental conditions:	**Temperature:** Not stated **Humidity:** **Air changes:** **Photoperiod:**
Acclimation:	Not stated

B. Study design and methods

1. Study experimentation dates - Start: Not stated End: Not stated

2. Animal assignment - Animals were assigned randomly to the test groups noted in Table 1. Copies of proprietary reports of toxicity studies performed by Monsanto Corporation were made available to the NTP for use in designing its glyphosate studies. The adrenergic agents, isoproterenol and propranolol, were administered by continuous subcutaneous infusion by osmotic mini-pumps. A day prior to starting treatment, all rats were anesthetized with methoxyflurane and the osmotic mini-pumps were implanted subcutaneously. Group 1 (negative control) was fed standard NIH-07 diet and implanted with pumps containing vehicle (sterile water + 0.1% ascorbic acid). Group 2 was fed NIH-07 diet containing glyphosate (5000 ppm) and implanted with vehicle pumps. Group 3 was fed 5000 ppm glyphosate-dosed feed and implanted with pumps containing the adrenergic antagonist propranolol. As a positive control, group 4 was given the adrenergic agonist, isoproterenol, by pump and fed normal diet. Group 5 animals (blocking controls) were implanted with both isoproterenol and propranolol and fed normal diet.

Table 1: Study Design[a]

Group	Feed	Pump
1	Control	Vehicle (water + 0.1% ascorbate
2	Glyphosate (5000 ppm)	Vehicle
3	Glyphosate (5000 ppm)	Propranolol (~ 1.2 mg/kg/day)
4	Control	Isoproterenol (~ 1.0 mg/kg/day)
5	Control	Isoproterenol + propranolol

3. Diet preparation and analysis - The report stated that glyphosate in diet was stable for at least 3 weeks at room temperature when stored in the dark. Further details regarding the concentration, stability, and homogeneity analysis of the test compound in the diet were not provided.

4. Statistics – The statistical methods below were used for all studies that are discussed in the NTP report.

"Two approaches were employed to assess the significance of pairwise comparisons between dosed and control groups in the analysis of continuous variables. Organ and body weight data, which are approximately normally distributed, were analyzed using the parametric multiple comparisons procedures of Williams (1971, 1972, 1986) and Dunnett (1955). Clinical pathology and hematology data, which typically have skewed distributions were analyzed using nonparametric multiple comparisons methods of Shirley (1977) and Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of dose-response trends and to determine whether a trend-sensitive test (Williams, Shirely) was more appropriate for pairwise comparisons than a test capable of detecting departures from monotonic dose response (Dunnett, Dunn). If the P-value from Jonchkheere's test was greater than or equal to 0.10, Dunn's or Dunnett's test was used rather than Shirley's or Williams's test. The outlier test of Dixon and Massey (1951) was employed to detect extreme values. No value selected by the outlier test was eliminated unless it was at least twice the next largest value or at most half of the next smallest value."

C. Methods

1. Observations - Animals were inspected twice daily for mortality/moribundity, and once a week for clinical signs of toxicity.

2. Body weight - Animals were weighed weekly.

3. Food consumption and compound intake - Food consumption was measured every other day.

4. Hematology and clinical chemistry – Hematology and clinical chemistry measurements were not conducted.

5. Sacrifice and pathology – After 14 days of treatment, the left parotid and submandibular/sublingual glands were removed and weighed separately, after which the glands were cut into small pieces, placed into a 2.5% glutaraldehyde/ 2.0% paraformaldehyde solution, and processed for electron microscopy. The right parotid and submandibular/sublingual glands were removed and placed in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H&E) and Alcian Blue (pH 2.5)-periodic acid Schiff (AB-PAS). The results were reviewed and evaluated through an NTP Pathology Review. The reported data represented a consensus of contractor and review pathologists.

II. RESULTS

A. Observations:

1. Clinical signs of toxicity – Rats receiving isoproterenol were hypoactive and had increased respiratory rates on day 1 following pump implantation, but were normal by the following day according to the study

report.

Increased incidence of softer and wetter feces was reported for the glyphosate-fed animals by day 7 of the study.

2. Mortality – All rats survived to the end of 14-day treatment period.

B. Body weight and weight gain - A summary of body weight gain data is provided in table 2. Body weight data were not reported. Decrease in body weight gains in the glyphosate-fed groups was noted compared to the other groups.

Table 2 Body weight (g) and body weight gain (g)[a]

Group (diet/pump)	Food consumption g/rat/day	Body weight gain (g)
1 (control diet/vehicle)	14.4	16.0 ± 2.9
2 (glyphosate/vehicle)	17.6	6.3 ± 2.0
3 (glyphosate/propranolol)	20.4	6.0 ± 2.4
4 (control diet/isoproterenol)	14.9	16.7 ± 1.6
5 (control diet/isoproterenol + propranolol)	15.0	17.5 ± 8.0

C. Food consumption – A summary of food consumption data is provided in table 2. The average daily food consumption was higher in the glyphosate-fed groups compared to the other groups.

G. Sacrifice and pathology -

1. Organ weight – Parotid and submandibular/sublingual organ weight data are summarized in table 3. Individual organ weight data was not provided in this study. Absolute parotid weight was increased in the group 2 (glyphosate-fed = 3-fold), group 3 (glyphosate-fed and adrenergic antagonist ~ 2.0 fold), and group 4 (isoproterenol given by subcutaneous pump = 54%) compared to group 1. Absolute submandibular/sublingual was increased in group 2 (~ 2.0 fold), group 3 (25%), and group 4 (24%) compared to group 1.

Table 3. Organ weights Data[a]

Group (diet/pump)	Parotid		Submandibular/sublingual	
	Absolute (mg)	Relative*	Absolute (mg)	Relative*
1 (control diet/vehicle)	126.2 ± 16.2	0.50 ± 0.08	209.7 ± 14.8	0.83 ± 0.04
2 (glyphosate/vehicle)	354.0 ± 37.5	1.47 ± 0.12	375.0 ± 26.3	1.56 ± 0.07
3 (glyphosate/propranolol)	245.0 ± 10.4	1.06 ± 0.06	261.0 ± 6.4	1.13 ± 0.04
4 (control diet/isoproterenol)	194.2 ± 15.6	0.76 ± 0.06	259.7 ± 10.6	1.03 ± 0.03
5 (control diet/isoproterenol + propranolol)	137.2 ± 19.1	0.55 ± 0.07	225.5 ± 7.8	0.91 ± 0.05

* mg/g body weight

2. Microscopic pathology - The histopathological findings are reported in Table 3. Increased incidence of lesions in the parotid gland was observed in the in all groups compared to group 1(control). Increased incidence of lesions was also observed in the submandibular gland of the group 2 (glyphosate + vehicle) and 3 (glyphosate + propranolol) animals. Parotid lesions consisted of cytoplasmic basophilic change, fine vacuolation, and swelling of acinar cells, diagnosed collectively as cytoplasmic alteration. A distinct gradation in the severity of these lesions was reported which was based on the extent of involvement and degree of tinctorial alteration and cell enlargement.

Parotid glands of glyphosate-treated animals were most severely affected. The parotid glands of all these animals were characterized by diffuse, intense basophilic change of acinar cells with clearly evident acinar enlargement, resulting in a relative reduction in the number of ducts present. Concurrently, the

cytoplasm of affected cells was finely vacuolated, and nuclei were hyperchromatic and displaced more basally by increased cytoplasmic secretory granules. In serial sections stained with Alcian Blue/Periodic acid Schiff (AB/PAS), areas of cytoplasmic alteration were seen to be associated with loss of PAS positive staining of secretory granules. Electron micrographs showed an increase in size and number of these secretory granules.

The lesions of the submandibular glands consisted of cellular and acinar swelling with a relative reduction in the number of duct profiles per field. Tinctorial change was less of a component of the submandibular lesion than in the parotid; with more acinar cells being paler than controls, with scattered individual cells or acini being more basophilic, imparting a mottled staining pattern to the tissue. AB-PAS reactivity was unchanged

No inhibitory effect of propranolol was noted in the incidence of the lesions in the submandibular glands of the glyphosate-treated animals compared to animals that were not exposed to propranolol (i.e. Group 3 vs. group 2). Animals receiving the adrenergic antagonist, propranolol, subcutaneously and concurrently with glyphosate-dosed feed did not exhibit high severity of these lesions.

Isoproterenol administration alone or with propranolol resulted in similar lesions in parotid gland that glyphosate produced with lower degree of severity.

Table 3 - Incidence and Severity of Cytoplasmic Alteration of the Parotid Salivary Glands (combined) following the 90 day treatment with glyphosate[a]

Group (diet/pump)	Parotid - Incidence/severity (mean severity)	Submandibular – Incidence	Sublingual – Incidence
1 (control diet/vehicle)	1/4 (1.0)*	0/4	0/3
2 (glyphosate/vehicle)	4/4 (4.0)	4/4	0/4
3 (glyphosate/propranolol)	3/4 (1.5)	4/4	0/2
4 (control diet/isoproterenol)	4/4 (2.7)	0/4	0/1
5 (control diet/isoproterenol + propranolol)	4/4 (2.0)	0/4	0/4

* Average severity grades for parotid gland lesions in affected animals, based on the following scale: 1 = focal change; 2 = multifocal, confluent change; 3 = diffuse change; 4 = diffuse change with intense basophilia and marked acinar swelling.

III. DISCUSSION

A. <u>Investigators' conclusions</u> - "In the 13-week studies, glyphosate did not affect survival of F344/N rats or B6C3F1 mice. Body weight gains were depressed in rats and mice at the 2 highest dose levels; weight gain depression was more severe in males than in females. Kubena et al (1981) reported that body weight gains were reduced (about 50%) in male and female chicks fed a diet containing 6080 ppm of the isopropylamine salt of glyphosate for 21 days, beginning at 1 day of age; the calcium and magnesium content of the tibiotarsus bone was increased compared to controls. There were no differences in body weights in chicks fed a dose of 608 ppm or lower. In the Kubena study (which did not mention feed palatability) and in our 13-week study, the possibility of reduced food intake in the high dose groups cannot be ruled out; more food tends to be spilled when it is not palatable, and our food consumption measurements did not account for scattered feed. Poor palatability of feed containing high concentrations of glyphosate is suggested by the finding that rats drank less water containing Roundup® at 10000 ppm or higher. Another possibility is that the higher concentrations of glyphosate in feed result in poor absorption of dietary components from the GI tract. However, if uncoupling of oxidative phosphorylation, as proposed by Olorunsogo et al. (1979) and Bababunni et al. (1979), is occurring as a result of glyphosate ingestion, then a reduction in weight gain for a given amount of food consumed would be expected.

It is noteworthy that the U.S. Environmental Protection Agency, after reviewing an unpublished 2-year carcinogenicity study of glyphosate in CD-1 mice, announced that there was "equivocal carcinogenic response, possibly causing a slight increase in the incidence of renal tubular adenomas in male mice at the highest dose tested (30000 ppm)." A carcinogenicity study in rats hast yet to be reviewed (Anonymous, 1991). In the present study, however, the salivary gland was identified as the sole target organ for glyphosate toxicity in both rats and mice. The lesion was diagnosed as cytoplasmic alteration of the acinar epithelial cells, consisting of increased basophilic staining and vacuolation of cytoplasm, and enlargement of cells and acini. This lesion was limited to the parotid gland in mice but affected both parotid and submandibular glands in rats; the sublingual gland was not affected. Salivary gland lesions are relatively uncommon in toxicity studies; however, both spontaneous and chemically-induced changes of a similar nature to those seen in the glyphosate study have been described. So called "basophilic hypertrophic foci" occasionally may be seen as a spontaneous lesion in the parotid gland or rats and mice (Chiu and Chen, 1986); however, these are infrequent and focal in nature. More extensive and diffuse basophilic and hypertrophic change has been described in subchronic studies with some chemicals, such as doxylamine (Jackson and Blackwell, 1988) and methapyrilene (Jackson and Sheldon, 1984). By far, the most extensive and detailed studies of these changes in salivary glands have been done with sympathomimetic agents -- for example, the adrenergic agonist, isoproterenol, which induces striking morphologic changes in salivary glands (Schneyer, 1962; Fukuda, 1968). As with glyphosate's effects on the salivary glands, isoproterenol affects the parotid and submandibular glands but not the sublingual. This is due to the fact that, in the rat, the acini of the parotid and submandibular are richly supplied with adrenergic fibers, while the sublingual gland is devoid of adrenergic innervation (Nordenfelt, 1967). Because glyphosate and isoproterenol are similar in both morphologic effects induced in the salivary glands and the gland specificity of those effects, it was hypothesized that glyphosate-related lesions were mediated through an adrenergic mechanism. A study was designed to test this hypothesis.

Two weeks' exposure to glyphosate by dosed feed resulted in marked increases in parotid and submandibular salivary gland weights. This effect on salivary gland weights is similar to that of isoproterenol, both as described in the literature (Schneyer, 1962) and as seen in the positive control of this study. Increased salivary gland weights were associated histologically with cytoplasmic alteration of acinar cells. This effect was more marked in the parotid than in the submandibular gland. In the parotid, the cytoplasmic changed induced by both glyphosate and isoproterenol was associated with a loss of the granules or a change in their chemical composition. The sublingual gland was not affected histologically by either glyphosate or isoproterenol, demonstrating target specificity of glyphosate- and isoproterenol-associated lesions to those salivary glands which are innervated by adrenergic fibers (Nordenfelt, 1967).

The effect of adrenoreceptor stimulation on parotid acinar cells has been described by ultrastructural and morphometric criteria to be increases in cell size, primarily due to increases in the number and size of secretory granules, as well as changes in the staining of these granules from electron dense to lucent, interpreted to represent a mucoid transformation of the cell (Schneyer, 1962; Henriksson, 1982; Carlsoo et al., 1984). These findings are identical to those found upon electron microscopic examination of parotid cells from animals treated with both glyphosate and isoproterenol in this study, the effects varying only in degree between the chemicals. Ultrastructural effects in the submandibular gland were similar between these compounds, though of a less well-defined nature, these effects consisted of cell enlargement due to accumulation of lucent or heterogeneous staining mucoid type granules, although it was not clear whether the serous or mucous cells of the acinus were being affected. This study led to the conclusion that the salivary gland effect is mediated through an adrenergic mechanism, as evidenced by (1) inhibition of the glyphosate-induced effect by the adrenergic antagonist, propranolol; (2) the similarity between the effects of glyphosate and the adrenergic agonist, isoproterenol; and (3) the specificity of those effects for salivary glands with adrenergic innervation.

The biologic significance of this finding is unknown. In addition to basophilic and hypertrophic morphologic changes of acinar cells, treatment with isoproterenol has been associated with increased cell proliferation in the parotid gland (Schneyer et al., 1967). This suggests that if glyphosate is acting through an adrenergic pathway, it may likewise induce hyperplasia in this gland, possibly predisposing it to neoplastic change; however, this is not considered likely, since spontaneous basophilic, hypertrophic foci of the parotid, as well as of the pancreas (an anatomically similar tissue) are not considered to be pre-neoplastic lesions. Moreover, there was no increased incidence in rats of salivary gland tumors in a 2-year study of methapyrilene (personal communication, Dr. I. Hirono, Fujita Gakuen Health University, Japan, May 17, 1991), a chemical which induced similar salivary gland lesions as glyphosate in subchronic studies."

B. <u>Reviewer comments</u> – The objectives of this study was to show that the salivary glands toxicity produced by glyphosate occur through the adrenergic pathway.

Increased salivary glands (parotid and submandibular) weights and parotid gland lesions diagnosed as cytoplasmic alteration was noted for glyphosate-fed group and a β-adrenergic receptor agonist (isoproterenol) administered group. Similar lesions were noted in the submandibular gland for glyphosate-fed group, but not the β-agonist (isoproterenol) administered group. A β-blocker (propranolol) showed some suppression of the severity of the parotid lesion when given concurrently with glyphosate. Administration of propranolol did not show a suppression of the incidence (e.g. presence) of this lesion in the parotid and submandibular glands when given concurrently with glyphosate. The rationale behind the selection of the doses for propranolol and isoproterenol were not discussed in the study report. The dose of propranolol compared to the dose of glyphosate will likely influence the suppression of the lesions in the salivary gland as there may be competitive binding/affinity to the receptor site. This could explain the reason that a pronounced suppression of the lesions in the salivary glands was not noted.

The study author concluded that glyphosate is acting through an adrenergic mechanism. The study reviewer disagreed with this conclusion because demonstrating that a compound could act through an adrenergic mechanism requires extensive data describing step by step process of how a compound perturbs such a pathway. In order to provide more convincing data that glyphosate has activity in the β-adrenergic receptors (e.g. through weight of evidence), other organs that are innervated with β- receptors should also be examined. Therefore, the following is a short list of major endpoints (i.e. empirical evidence) that should be observed concurrently with salivary glands effects when test animals are exposed to a β- receptor agonist:

- Increased heart rate (pulse rate)
- Increased respiration rate
- Increased blood pressure due to renin release from the kidneys
- Relaxed bronchioles
- Dilate pupils of eyes
- Uterine relaxation
- Increased blood sugar
- Thickened secretions from the salivary glands

The thickened secretions from the salivary glands were perhaps shown in this study indirectly by the electron micrographs of the salivary glands that indicated an increase in size and number of secretory granules within the acinus cells. The examination of the other abovementioned endpoints are generally not required in the toxicity studies (except glucose levels = increased blood sugar, and dilated pupil of eyes in the clinical observation of these studies). Therefore, special mechanistic studies can be conducted to examine these endpoints in order to provide convincing evidence that glyphosate has activity at β-

receptors.

Hypertrophy, acinar enlargement, cytoplasmic alterations, reduced number of ducts and increase in size and number of secretory granules are noted in the parotid and/or submandibular glands of animals exposed to glyphosate in number of studies besides this one (a 28-day study in rats in three strains of rats, two 90-day studies in SD and F344 rats, a 90 day study in mice, a two year study in SD rats, and two generation reproduction toxicity in SD rats). The effect is more pronounced in F344 strains compared to other strains of rats. These studies examined the effects on the structures of the salivary glands compared to the effect on the function of these organs. The functions of saliva (salivary glands) fall into three general categories of digestion, lubrication, and protection. The digestive actions are the results of the two enzymes; the amylase and lipase that digest carbohydrates and fat respectively. The lubricating properties of saliva are due primarily to its mucus content. Protection properties of saliva are through the serous secretions that dilute and buffer harmful substances. The parotid glands are made up only of serous cells while the submandibular and sublingual glands are made up of serous and mucous secreting cells. The acinar cells secrete the initial salivary gland fluid, consisting of electrolytes, mucus, and enzymes. From the acinus, saliva passes relatively unchanged through the ducts. The basement membranes of acini and ducts are covered in part by specialized contractile cells. The contraction of these cells occur when salivary secretion is stimulated (by parasympathetic and sympathetic nervous system among other factors e.g. food in the mouth) and results in the release of saliva into the mouth.

The salivary gland effects from the administration of glyphosate were noted in short and long term studies and these lesions did not turn into neoplastic ones in the long term studies. The reduced number of ducts in this study was noted. This study did not specify whether the reduced number of ducts was due to hypertrophy (same absolute number of ducts compared to control animals) or there was an absolute decrease in the number of ducts. With lack of data on the function of salivary glands because of these effects, the toxicological significance of such these findings are unknown. However, a NOAEL/LOAEL should be established on these effects (especially combined with increased salivary glands weights) until such data are available to discern the toxicological adversity. The IPCS JMPR/WHO (2004) also concluded that this finding is of unknown toxicological significance however establishing the ADI/NOAEL on this effect and requesting further data for clarification.

Overall, this study is considered acceptable and classified as appropriate for qualitative use for hazard characterization of glyphosate. It is recognized that raw data was not provided in the study report; however, this report provided a relatively comprehensive understanding of the conditions under which the study was conducted and of the data generated by the study. In addition, the effects noted in this study (e.g. salivary glands toxicity) are aligned with other lines of evidence throughout the glyphosate toxicology database (e.g. other short and long term studies have reported salivary gland toxicity).

Reviewer # 2032 **Date** Oct 29, 2012

Study type: Bacterial reverse mutation assay - (*Salmonella typhimurium*; *E. coli*); OPPTS 870.5100[8]; OECD 471 (formerly OECD 471 & 472).

Test Material (purity): Glyphosate (98% a.i.)

Synonyms: N-phosphomethyl-glycine

Citation: Li, A.P. and Long, T.J. An evaluation of the genotoxic potential of glyphosate. Fundamental and Applied Toxicology 10:537-546. Institute of Environmental Toxicology, Tokyo. Laboratory report number unspecified. Journal article date: 10-11-1987. DACO 4.5.4.

Sponsor: Monsanto Co.

MRID: NA

Executive Summary: In a reverse gene mutation (plate-incorporation) assay in bacteria, strains TA1535, TA1537, TA1538, TA100 and TA98 of *S. typhimurium* were exposed to glyphosate, (98% a.i.), in water at concentrations of 10, 50, 100, 500 and 1000 µg/plate in the presence and absence of mammalian metabolic activation (S9). The tryptophan-*hcr* strain of *E.coli* WP2 was used to test for glyphosate mutagenicity in the presence and absence of S9 using plate-incorporation technique, simultaneously with *Salmonella*/histidine reversion assay, using the same doses.

Glyphosate was tested up to concentration of 5000 µg/plate, showing cytotoxicity at the highest dose only in WP2 hcr strain in reversion assay. No statistically significant induction of revertants above solvent control levels and significant dose-response relationship were observed. The positive controls induced the appropriate responses in the corresponding strains. **There was no evidence of induced mutant colonies over background with glyphosate treatment.**

This study is classified as acceptable. This study satisfies the requirement for Test Guideline OPPTS 870.5100[1]; OECD 471 for mutagenicity (bacterial reverse gene mutation) data.

Compliance: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were not available since this was a published journal article.

[8]870.5100 - Reverse mutation E. coli WP2 and WP2uvrA; S. typhimurium TA 97, TA98, TA100, TA1535, TA1537
870.5140 - Gene mutation Aspergillus nidulans
870.5250 - Gene mutation Neurospora crassa

I. Materials and Methods

A. Materials

1. **Test material:** Glyphosate
   Description: NA
   Lot/Batch #: XHJ-64
   Purity: 98% a.i.
   CAS #: NA

   Solvent used: water

2. **Control materials:**
   Negative: water
   Solvent (final conc'n):
   Positive: Nonactivation:
   2-Aminoanthracene 10 µg/plate all strains
   and
   0.1 µg AF-2/plate for TA98, 0.05 µgAF-2/plate for TA100, 50 mgp-propiolactone for TA1535, 200 µg 9-aminiacridine/plate for TA1537, 50 µg2-nitrofluorene/plate for TA1538 and 0.25 µg AF-2/plate for WP2 hcr.

   Activation:
   2-Aminoanthracene 10 µg/plate all strains
   Other (list):

3. **Activation:** S9 derived from

x	Induced	x	Aroclor 1254	x	Rat	x	Liver
	Non-induced		Phenobarbitol		Mouse		Lung
			None		Hamster		

Aroclor 1254-induced rat liver homogenate supernatant (S9) was prepared according to the methods described by Ames and coworkers (Ames, B.N., McCann, J. and Yamasaki, E., Mut. Res., 31:347-364, 1975). No further information about determination of the activity and storage of the S9 fraction was provided.

4. **Test organisms:** S. typhimurium strains

	TA97	x	TA98	x	TA100		TA102		TA104
x	TA1535	x	TA1537	x	TA1538		list any others		

Properly maintained?	Yes	No
Checked for appropriate genetic markers (rfa mutation, R factor)?	Yes	No

5. **Test compound concentrations used:**
   Non-activated conditions: 10, 50, 100, 500, 1000 or 5000 µg/plate
   Activated conditions: 10, 50, 100, 500, 1000 or 5000 µg/plate
   All doses of the test substance and controls were plated in the main assay in 2 replicates.

B. Test performance

1. Type of Salmonella assay

x standard plate test
_ pre-incubation (minutes)
_ "Prival" modification (i.e. azo-reduction method)
_ spot test

2. Protocol – Glyphosate was tested for mutagenicity in five strains of *Salmonella typhimurium,* including TA1535, TA1537, TA1538 and TA98 for detection of frame-shift mutation and in TA100 for detection of base-pair substitution using plate-incorporation assay, with or without activation. Glyphosate was dissolved in water and tested at doses of 10, 50, 100, 500, 1000 or 5000 µg/plate. The tryptophan-*hcr* strain of *E.coli* WP2 was used to test for glyphosate mutagenicity in the presence and absence of S9 using plate-incorporation technique, simultaneously with *Salmonella*/histidine reversion assay, with the same doses. Distilled water was used as the solvent control. 2-Aminoanthracene (10 µg/plate) was used as the promutagen positive control for all five strains and WP2-hcr. Direct-acting positive controls included β-propiolactone (50 µg/plate), AF-2 (2-(2-furyl)3-(5-nitro-2- furyl) acrylamide), (0.05-0.25 µ/plate), 9-aminoacridine (200 µg/plate), and 2-nitrofluorene (50 µg/plate). The incubation timing was not specified.

3. Statistics – The statistical-significance of the dose-response relationship was determined using linear-regression analysis without further data transformation at the significance level of $p \leq 0.05$.

4. Evaluation criteria – Not specified

II. Results

A. Preliminary cytotoxicity assay – No preliminary cytotoxicity assay was included with the study.

B. Mutagenicity assay – the highest concentration tested in WP2 hcr was cytotoxic, and no colonies were noted. No statistically significant induction of revertants above solvent control levels, and significant dose-response relationship was observed with glyphosate treatment. All the positive controls yielded the expected results. It was concluded that the test system was capable of detecting base-pair substitutions and frame-shift mutagens and that the metabolic activation system was functioning properly. Because the main assay was done in two replicates, no standard deviation was generated.

Table 1. Revertant colonies per plate (2 trials), standard plate test

	Not activated with S9							
Dose (µg/plate)	0	10	50	100	500	100	5000	Positive
TA98	24, 23	27, 28	33, 40	20, 20	31, 24	21, 23	10, 3	>3000, >3000
TA100	167, 129	130, 160	151, 159	143, 160	118, 143	87, 120	58, 87	1024, 1150
TA1535	6, 14	2, 5	5, 5	4, 5	3, 1	9, 12	6, 6	315, 358
TA1537	9, 10	3, 7	5, 6	8, 8	11, 9	10, 10	3, 3	1024, 1150
TA1538	10, 13	17, 24	15, 15	17, 24	7, 15	18, 12	6, 7	>3000, >3000

WP2 hcr	20, 24	22, 21	12, 25	18, 20	21, 26	15, 18	*, *	1672, 2272

*Inhibition of bacterial growth was observed
Positive controls included: 0.1µg AF-2/plate for TA98, 0.05 µgAF-2/plate for TA100, 50µgp-propiolactone for TA1535, 200µg 9-aminiacridine/plate for TA1537, 50µg2-nitrofluorene/plate for TA1538 and 0.25µg AF-2/plate for WP2 hcr.

Table 2. Revertant colonies per plate (2 trials), standard plate test

	Activated with S9							
Dose (µg/plate)	0	10	50	100	500	100	5000	Positive (2-aminoanthracene)
TA98	22, 16	19, 23	21, 26	9, 20	19, 26	15, 23	19, 22	>3000, >3000
TA100	139, 140	110, 135	123, 131	129, 115	138, 111	97, 88	51, 36	>3000, >30000
TA1535	6, 5	4, 1	9, 5	5, 7	3, 3	11, 4	5, 7	376, 335
TA1537	7, 5	3, 3	7, 9	11, 6	12, 5	11, 7	6, 3	370, 388
TA1538	8, 11	16, 11	13, 17	18, 14	15, 7	20, 11	11, 15	>3000, >3000
WP2cr	17, 22	25, 18	27, 22	33, 17	28, 30	29, 24	25, 34	98, 79

Positive controls included 2-aminoanthrace (all strains)

III. Discussion

A. <u>Investigators' conclusions</u> – "*Salmonella typhimurium* strains TA1535, TA100, TA1537, TA1538 and TA98 were treated with 10 to 5000 µg/plate of glyphosate in both the presence and absence of S9. No statistically significant induction of revertants above solvent control levels and significant dose-response relationship were observed. The positive controls yielded the expected positive responses." Same results were noted for WP2 reversion assay. Glyphosate was considered nongenotoxic on the basis of results.

B. <u>Reviewer comments</u> - The study author's conclusions are acceptable. Cytotoxicity was noted at the high dose in WP2 hcr strain. The test substance did not show a dose-related or biologically relevant increase in the number of revertant colonies/plate over the negative control with any Salmonella typhimurium or Escherichia coli tester strains trial in the presence or absence of S9 metabolic activation. **There was no evidence of induced mutant colonies over background with glyphosate treatment.**

C. <u>Study deficiencies</u> – A summary of the study with data was only available in a form of journal article. Some protocol details and evaluation criteria were not specified. As well, only two replicates instead of three were performed.

Reviewer # 2032 **Date** Dec 31 2012

Study Type: In vitro mammalian cell assay - CHO cells; OPPTS 870.5300; OECD 476.

Test Material (purity): Glyphosate (98% a.i.)

Synonyms: N-phosphomethyl-glycine

Citation: Li, A.P. and Long, T.J. 1988.An evaluation of the genotoxic potential of glyphosate. Fundamental and Applied Toxicology 10:537-546. Institute of Environmental Toxicology, Tokyo. Laboratory report number unspecified. DACO 4.5.5.

Sponsor: Monsato Co.

MRID: NA

Executive Summary: In a mammalian cell gene mutation assay HGPRT, CHO cells cultured *in vitro* were exposed to glyphosate, (98% a.i.), in Ham's F12 medium at concentrations of 2-25 mg/mL in the presence and absence of mammalian metabolic activation (1-10%), in two independent experiments.

Glyphosate was tested up to cytotoxic concentrations (i.e., 20g/mL). The positive controls induced the appropriate response. **There was no evidence of induced mutant colonies over background with glyphosate treatment.**

This study is classified as acceptable. This study satisfies the requirement for Test Guideline OPPTS 870.5300, OECD 476 for *in vitro* mutagenicity (mammalian forward gene mutation) data; however some deficiencies are also present.

Compliance: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were not available, since this was a published journal article.

I. Materials and Methods

A. Materials

1. **Test material:** Glyphosate
   Description: NA
   Lot/Batch #: XHJ-64
   Purity: 98% a.i.
   CAS #: NA

   Solvent used: Medium without serum

2. **Control materials:**
   Negative: DMSO (1%) and pyrene (50µM)
   Solvent (final conc'n): 0.1 N sodium hydroxide 1% of final volume
   Positive: Non activation: ethyl methanesulfonate (EMS, 200µg/mL)
   Activation: benzo[a]pyrene (BaP, 2µg/mL)

3. **Activation:** S9 derived from

	Induced	X	Aroclor 1254	X	Rat	X	Liver
	Non-induced		Phenobarbitol		Mouse		Lung
			None		Hamster		Other
			Other		Other		

Aroclor 1254-induced rat liver homogenate (S9) commercially purchased from Litton Bionetics was used as exogenous activation system. The S9-cofactor, consisting of 50 mM sodium phosphatate (pH 7.5), 4 mM NADP, 5mM glucose-6-phosphate, 30 mM KCl, 10 mg MgCl2, and different amounts of liver S9 was used.

4. **Test cells:** mammalian cells in culture

	mouse lymphoma L5178Y cells		V79 cells (Chinese hamster lung fibroblasts)
x	Chinese hamster ovary (CHO) cells		list any others

Media: Ham's F12 medium with or without serum (dialyzed newborn calf)

Properly maintained?		Yes	x	Unspecified
Periodically checked for Mycoplasma contamination?		Yes	x	Unspecified
Periodically checked for karyotype stability?		Yes	x	Unspecified
Periodically "cleansed" against high spontaneous background?		Yes	x	Unspecified

5. **Locus Examined:**

		Thymidine kinase (TK)		**Hypoxanthine-guanine-phosphoribosyl transferase (HGPRT)**		**Na^+/K^+ ATPase**
Selection agent:		bromodeoxyuridine (BrdU)		8-azaguanine (8-AG)		ouabain
		fluorodeoxyuridine (FdU)	x	6-thioguanine (6-TG)		
		trifluorothymidine (TFT)				

6. **Test compound concentrations used**: First and second experiment
   Nonactivated conditions: 5, 17.5, 22.5 mg/ml
   Activated conditions: 5, 17.5, 22.5 mg/ml with 1, 2, 5 or 10% with 1 mL of S9/cofactor mixture to a final volume of 5 mL

   Non activated conditions: 2-20mg/mL mg/ml

Activated conditions: 5-25 mg/L with 5% of S9/cofactor mixture

B. Test performance

1. Cell treatment -
CHO cell line (K1BH4) was originally obtained from Dr. A. W. Heise of Oak Ridge National Laboratory. For cytotoxicity determination, the cells were seeded in 25 cm^2 plastic culture flasks at $0.5x10^6$ cells/flask in growth medium for 18-24 hours prior to treatment. The medium was then changed to 2.5 mL Ham's F12 medium without serum, with or without S9 and an equal volume of 2 x solutions of glyphosate (dissolved directly in medium without serum) was then added. The tubes were incubated for 3 hours at 37.5±2ºC and the treatment medium was discarded and cells were washed with Hank's balanced salt solution. Cells were then removed from flasks by tryptonization and counted.

Three samples of 200 cells were plated for determination of cloning efficiency. The plates were incubated for 7-9 days and developed colonies were fixed with 70% methanol, stained with 10% Giemsa and counted by hand.

Cloning efficiency (C.E.) = # of colonies/# cells plated
Relative survival (R.S.) = C.E. (treated)/ C.E. (control)

For mutagenicity determination, the cells were exposed to test compound just as above, with negative/solvent or positive controls for 18-24 hours (non-activated or activated). Two independent mutagenicity experiments were performed. In the first experiment glyphosate toxicity was tested at 5, 17.5, 22.5 mg/mL ± 1, 2, 5 and 10% S9 (v/v). In the second experiment, glyphosate concentrations of 2-20 mg/mL –S9 or with 5 and 25 mg/mL +5% S9 were used.

After washing, 10^6 cells were subcultured every 2-3 days for 7-9 days (expression period) before cell selection using hypoxanthine-free Ham's F12 medium, supplemented with 10 µM 6TG and 5% dialyzed newborn calf serum.

After expression, $2x10^5$ cells/dish (5 dishes/ group) were cultured for 8-12 days in in 8ml selection medium to determine numbers of mutants and 200 cells/dish (3 dishes/group in 2mL medium) were cultured for 8-12 days without 6GT selective agent to determine cloning efficiency. The colonies which developed were fixed, stained and counted, with results expressed as Mutant Frequency (M.F.)

M.F>= # of mutant colonies/# cells plated x 1/C.E.

2. Statistics – "Mutagenicity data were analyzed according to statistical method of Snee and Irr (1981), designed specifically for CHO/HGPRT mutation assay. Mutant frequency values were transformed according to the equation $Y=(X+1)^{0.15}$, where Y=transformed mutant frequency, and X=observed mutant frequency. Student's t test was then used to compare treatment data to solvent control data. The Snee and Irr analysis also allowed the determination of the dose-response relationship as linear, quadratic, or higher order. A computer program obtained from Dr. Joe Irr (Dupont) and incorporated into the Monsato computer by Alan Dickson (Monsato) was used."

3. Evaluation criteria – The indexes below were used for calculation, however evaluation criteria were not discussed.

Cloning efficiency (C.E.) = # of colonies/# cells plated
Relative survival (R.S.) = C.E. (treated)/ C.E. (control)
M.F>= # of mutant colonies/# cells plated x 1/C.E.

II. Reported Results

A. Preliminary cytotoxicity assay – Significant cytotoxicity was observed for 20 mg/mL of glyphosate and 25 mg/mL glyphosate concentrations in the absence and presence of S9, respectively.

B. Mutagenicity assay – Two individual experiments were performed. The glyphosate treatment groups did not have statistically significant higher mutant frequency than the negative control (solvent) and no statistically significant dose-response. The positive controls EMS and BaP yielded the expected positive results. A decrease in mutagenicity of BaP was noted with increasing S9 concentrations in the first experiment. The decrease was explained as a result of detoxification (e.g. GSH conjugation) of the active BaP metabolites by the liver S9.

Table 1. Mutant frequency (group mean), first test

	Not activated with S9				
Dose (mg/mL)	0	5	17.5	22.5	Positive (EMS 0.2 mg/mL)
Cytotoxicity[a]	1.00	1.10	0.64	0.11	0.92
Mutagenicity/Mutant frequency[b] x 10^{-6}	7.4	7.1	14.4	5.3	163.7*

Table 2. Mutant frequency (group mean), first test

	Activated with 1% S9				
Dose (mg/mL)	0	5	17.5	22.5	Positive (BaP)
Cytotoxicity[a]	1.00	1.12	0.69	0.25	0.74
Mutagenicity/Mutant frequency[b] x 10^{-6}	5.9	4.3	11.6	15.3	353.1*
	Activated with 2% S9				
Dose (mg/mL)	0	5	17.5	22.5	Positive (BaP)
Cytotoxicity[a]	1.00	1.00	0.77	0.44	0.49
Mutagenicity/Mutant frequency[b] x 10^{-6}	7.1	8.6	8.1	10.2	185.9*
	Activated with 5% S9				

Dose (mg/mL)	0	5	17.5	22.5	Positive (BaP)
Cytotoxicity[a]	1.00	1.00	0.77	0.44	0.49
Mutagenicity/Mutant frequency[b] x 10^{-6}	4.4	6.4	6.9	8.5	121.2*
	Activated with 10% S9				
Dose (mg/mL)	0	5	17.5	22.5	Positive (BaP)
Cytotoxicity[a]	1.00	1.02	0.37	0.18	0.35
Mutagenicity/Mutant frequency[b] x 10^{-6}	9.1	10.5	16.3	9.7	95.3*

Table 3. Mutant frequency (group mean), second test

	Not activated with S9						
Dose (mg/mL)	0	2	5	10	15	20	Positive (EMS 0.2 mg/mL)
Cytotoxicity[a]	1.0	0.99	0.93	0.90	1.04	0.38	0.92
Mutagenicity/Mutant frequency[b] x 10^{-6}	11.3	3.5	11.3	10.8	20.8	10.1	135.4*

[a] survival relative to solvent control (average of triplicate treatment)
[b] Mutants per 10^6 clonable cells (average of duplicate treatments)
* $p \leq 0.05$

Table 4. Revertant colonies (group mean), second test

	Activated with S9					
Dose (mg/mL)	0	10	15	20	25	Positive (BaP)
Cytotoxicity[a]	1.00	1.15	0.99	1.13	0.46	0.47
Mutagenicity/Mutant frequency[b] x 10^{-6}	11.3	5.7	13.1	9.9	13.1	76.8*

[a] survival relative to solvent control (average of triplicate treatment)
[b] Mutants per 10^6 clonable cells (average of duplicate treatments)
* $p \leq 0.05$

III. Discussion

A. <u>Investigators' conclusions</u> – "As observed in the first experiment, none of the glyphosate treatment groups had a statistically significant higher mutant frequency than the solvent control and no statistically significant dose-response relationship (linear, quadratic, or higher-order) between dose and mutant frequency was observed."

B. <u>Reviewer comments</u> – The reviewer is in agreement with the investigators' conclusions. The positive controls induced mutant colonies over background. **There was no evidence of induced**

mutant colonies over background with glyphosate treatment of CHO cells.

C. <u>Study deficiencies</u> – Standard deviation was not provided for the data. No information was provided about upkeep of medium and cells karyotype specificity. No information was provided about evaluation criteria for the data.

Reviewer # 2032 **Date** Dec 31 2012

Study Type: In vivo cytogenetics - micronucleus assay in rat; OPPTS 870.5395; OECD 474.

Test Material (purity): Glyphosate (98% a.i.)

Synonyms: N-phosphomethyl-glycine

Citation: Li, A.P. and Long, T.J. An evaluation of the genotoxic potential of glyphosate. Fundamental and Applied Toxicology 10:537-546. Institute of Environmental Toxicology, Tokyo. Laboratory report number unspecified. Journal article date: 10-11-1987. DACO 4.5.7.

Sponsor: Monsato Co.

MRID: NA

Executive Summary: In a Sprague Dawley rat bone marrow micronucleus assay, animals (18sex/dose) were treated ip with glyphosate (98% a.i.) at doses of 0, 1000 mg/kg bw. Bone marrow cells were harvested at 6, 12, 24 post-treatment (6/sex/time). The vehicle was HBSS.

There were no signs of toxicity during the study. Glyphosate was tested at an adequate dose (based on limit dose of 1000 mg/kg). The positive control induced the appropriate response. **There was not a significant increase in the frequency of percentage of chromatic aberrations in bone marrow after any treatment time with glyphosate, as compared to the negative control.**

This study is classified as acceptable. This study satisfies the requirement for Test Guideline OPPTS 870.5395; OECD 474 for in vivo cytogenetic mutagenicity data.

Compliance: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were not provided since this is a published journal article.

I. Materials and Methods

A. Materials

1. **Test material:** Glyphosate
   Description: NA
   Lot/Batch #: XHJ-64
   Purity: 98% a.i.
   CAS #: NA

   Solvent used: Hank's balanced salt solution (HBSS) neutralized to 7.0 pH with 1N sodium hydroxide

2. **Control materials:**

Negative control		**Final Volume:**	**Route:**
Vehicle:	Solvent HBSS	**Final Volume: NA**	**Route: NA**
Positive control :	cyclophosphamide	**Final Dose(s): 25 mg/kg**	**Route: ip**

3. **Test animals:**

Species:	Rats
Strain:	Sprague Dawley
Age at study initiation:	NA
Weight at study initiation:	NA
Source:	NA
No. animals used per dose	18 males; 18 females
Properly maintained?	NA

4. **Test compound administration (ip):**

	Dose Levels	Final Volume	Route
Preliminary: **Main Study:**	1000 mg/kg bw	NA	ip

B. Test performance

1. Treatment and sampling times (n/sex):

a. **Test compound:**

Dosing:	n	once	n	once		
Sampling (after last dose)	6	6 hr	6	12 hr	6	24 hr

b. **Negative and/or vehicle control:**

Dosing:	n	once	n	once		
Sampling (after last dose):	6	6 hr	6	12 hr	6	24 hr

c. **Positive control:**

Dosing:	n	once	n	once		
Sampling (after last dose)	6	6 hr	6	12 hr	6	24 hr

2. **Tissues and cells examined:**

Bone marrow	30 cells/animal; 300 cells/treatment/time period
No. of polychromatic erythrocytes (PCE) examined per animal:	NA
No. of normochromatic erythrocytes (NCE; more mature RBCs) examined per animal:	NA

3. Details of slide preparation – Six animals of each sex from treatment and control groups were terminated at 6, 12 and 24 hours post-treatment. Two hours prior to termination, colchicine (2 mg/kg) was administered to all animals to arrest cells in metapahase. The bone marrow was collected from both femurs of each animal and processed for slide preparation. When possible, 50 cells per animal (~300 cells/treatment per time period) were examined for chromosome aberrations.

4. Evaluation criteria – No evaluation criteria were specified, including lack of differentiation between polychromatic and normochromatic erythrocytes.

5. Statistics – "Student's t test was used to determine the statistical significance of the difference between treatment groups (test chemical and positive control groups) and the vehicle control group, using a significance level of $p \leq 0.05$."

II. Reported Results

A. Preliminary toxicity assay – no information was provided.

B. Micronucleus assay – No general toxicity was observed for the male and female rats after glyphosate treatment at various time points. In a separate study, radiolabelled glyphosate was shown to reach the bone marrow, with a peak level at 0.5 hours after ip dosing and half time of elimination in excess of 7.6 hours. The ratio of PCE/NCE was not provided. Chromatid-type aberrations were observed in both the negative control and glyphosate treatment groups at low frequency. No statistically significant increases in chromosome aberrations or achromatic lesions (gaps) were observed in glyphosate treatment group as compared to the negative control, at any time tested. The positive control showed anticipated results, with high frequencies of chromosomal aberrations. No explanation was provided why so few female cells were collected for the positive control at 24 hour time period. The percent of aberrant cells was comparable at different time points in the glyphosate group, and slightly higher in the female animals.

Table 1. Chromatid aberrations observed (percent with aberrations) after 6 hour sampling time

Dose (mg/mL)	Solvent control M	Solvent Control F	Glyphosate 1000 M	Glyphosate 1000 F	Positive Control M	Positive Control F
Number of cells	300	300	300	300	NA	NA
Normal cells	296	292	293	291	NA	NA
Chromatid deletions	3	4	3	3	NA	NA
Chromatid interchanges	1	0	0	0	NA	NA
Chromatid intrachanges	0	0	0	0	NA	NA
Achromatic lesions	0	5	6	6	NA	NA
% aberrant cells	1.3	2.7	2.3	3.0	NA	NA

Table 2. Chromatid aberrations observed (percent with aberrations) after 12 hour sampling time

Dose (mg/mL)	Solvent control M	Solvent Control F	Glyphosate 1000 M	Glyphosate 1000 F	Positive Control M	Positive Control F
Number of cells	300	275	300	277	NA	NA
Normal cells	297	271	294	270	NA	NA
Chromatid deletions	1	1	3	2	NA	NA
Chromatid interchanges	0	0	0	0	NA	NA
Chromatid intrachanges	0	0	0	0	NA	NA
Achromatic lesions	2	1	3	6	NA	NA
% aberrant cells	1.0	1.5	2.0	2.5	NA	NA

Table 3. Chromatid aberrations observed (percent with aberrations) after 24 hour sampling time

Dose (mg/mL)	Solvent control M	Solvent Control F	Glyphosate 1000 M	Glyphosate 1000 F	Positive Control M	Positive Control F
Number of cells	300	265	192	300	256	21
Normal cells	296	259	190	289	148	16
Chromatid deletions	1	3	2	5	217	14
Chromatid interchanges	0	0	0	0	76	1
Chromatid Intrachanges	0	0	0	0	6	0
Achromatic lesions	3	5	0	6	34	3
% aberrant cells	1.3	2.3	1.0	3.7	**42.2***	**23.8***

* $p<0.05$

III. Discussion

A. <u>Investigators' conclusions</u> – "Chromatid-type aberrations were observed in both the solvent control and glyphosate treatment groups at low frequencies. Chromatid deletions, the most frequent category, was observed at a frequency of approximately 1%. No statistically significant increases in either chromosomal aberrations or achromatic lesions (gaps) were observed in the glyphosate-treated groups at any time point studied. The expected high frequencies of chromosomal aberrations were observed for the positive control groups."

B. <u>Reviewer comments</u> – The reviewer finds investigators' conclusions acceptable. Chromatid-type aberrations were observed at comparable frequency in both the solvent control and glyphosate treatment groups, indicating no increased induction by treatment. The positive control group induced a higher frequency of chromosomal aberrations over solvent control, as expected. **There was not a significant increase in the frequency of chromosomal aberrations in bone marrow after any treatment time with glyphosate.**

C. <u>Study deficiencies</u> – Only one dose of glyphosate was provided. The positive control data was not tabulated for the 6 and 12 hour time period. No evaluation criteria were specified,

including lack of differentiation between polychromatic (PCE) and normochromatic (NCE) erythrocytes and the number of NCE/PCE was not provided. Animal toxicity in the positive control was not noted. Some details of the protocol were not provided.

Reviewer # 1912 **Date** November 1, 2012

Study Type: Metabolism – Rat; OPPTS 870.7485 (Tier I & Tier II); OECD 417 (5.1.1/2).

Test Material (purity): Glyphosate (95% a.i.)

Synonyms: Glyphosate, technical grade; Glycine, N-(phosphonomethyl); N-phosphono-methyl glycine; N-(phosphonomethyl)glycine; MON0573; MON 2139.

Citation: Toxicokinetics of glyphosate and its metabolite aminomethyl phosphonic acid in rats. Department of Toxicology and Pharmacology, Faculty of Veterinary Medicine, Complutense de Madrid, 28040 Madrid, Spain. July 14, 2009

Sponsor: N/A

MRID: N/A

Executive Summary: In a time-course toxicokinetic study, glyphosate (95% a.i.) was administered to adult male Wistar rats by a single oral dose of 400 mg/kg bw via gavage or a single intravenous (i.v.) dose of 100 mg/kg bw (n = 80/exposure route).

Glyphosate was slowly and poorly absorbed orally. The absorption half-life was calculated to be 2.29 hours while the maximal plasma concentration was determined to be 4.64 µg/ml and time to maximal plasma concentration was determined to be 5.16 hours after the oral administration of glyphosate. The oral bioavailability of glyphosate was 23.21%. Glyphosate was also not extensively metabolized in rats. Aminomethyl phosphonic acid (AMPA) is the main metabolite which represented 6.49% of the parent plasma concentrations. The rate of elimination of AMPA ($T_{1/2\beta}$ = 15.08h) after oral glyphosate administration was similar to that of glyphosate ($T_{1/2\alpha}$ = 14.38).

After i.v. administration of 100 mg/kg bw, the distribution phase of glyphosate was fast ($T_{1/2\alpha}$ = 0.345 hr) and with a high volume of distribution at steady state (V_{ss} = 2.99 L/kg) glyphosate is extensively distributed in extravascular tissues. The two compartment model was the best fit for both groups to establish the toxicokinetic characteristics. The values of apparent volume of distribution in the second compartment were 2.39 and 2.32 L/kg after i.v. and oral administration, respectively.

The elimination half-life calculated after i.v. administration was 9.99 hours. The elimination half-life of glyphosate increased by 44% (to 14.38 hr) after oral administration compared to the i.v. administration.

Although this study was not conducted according the guideline requirement for a metabolism study (OPPTS 870.7485; OECD 417), it is considered acceptable and classified as appropriate for qualitative use for hazard characterization of glyphosate.

Compliance: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were not provided since this was a published journal article.

I. MATERIALS AND METHODS

A. Materials

Test material:	Glyphosate	
Purity:	95% a.i.	
Lot/Batch #:	Not provided	
CAS #:	107-83-6	
Vehicle:	Corn oil (oral) or glycerol formal (i.v.)	
Positive control:	None	
Preparation of dosing solutions:	Volume of 0.5 ml corn oil/rat (oral) or 0.1 ml glycerol formal (i.v.)	
Test species:	Rat	
Strain:	Male Wistar	
Age at start:	Not provided	
Weight at start:	200-210 g	
Source:	Charles River Inc, Margate, Kent, UK	
Housing:	Polycarbonate cage	
Diet:	A04 rodent diet, Panlab SL was provided *ad libitum*	
Water:	*ad libitum*	
Environmental conditions:	**Temperature:**	22±2°C
	Humidity:	50±10%
	Air changes:	Not stated
	Photoperiod:	12 hours dark/12 hours light
Acclimation:	Not provided	

B. Study Methods:

1. Study experimentation dates - Start: NA End: NA

2. Group Arrangements - Animals were assigned to the test groups noted in Table 1. The study did not mention that the animals were randomly assigned to the test groups.

Table 1: Dosing groups for pharmacokinetic studies for glyphosate

Test Group	Dose (mg/kg bw)	Number	Time of Sac (n=8 at each time) in HR
Oral (Gavage)	400	80♂	0.25, 0.5, 1, 2, 4, 6, 8, 10, 12, and 24 hr
Intravenously	100	80♂	0.25, 0.5, 1, 2, 4, 6, 8, 10, 12, and 24 hr

3. Dosing and sample collection – Eighty male rats were dosed orally via gavage and while the other 80 male rats received glyphosate intravenously via a single injection of 100 mg/kg bw into the lateral tail vein (in 0.1 ml glycerol formal/rat). Animals dosed orally were fasted for 12 hours prior to dosing. At each time point after dosing, 8 animals per group were sacrificed by cervical dislocation and then exsanguinated. Blood samples were withdrawn and collected in heparin tubes.

a. Pharmacokinetic studies – The plasma concentration of test material (glyphosate or AMPA) were measured by high performance liquid chromatography. Fluorescence agent 9-fluorenylmethylchloroformate (FMOC-Cl) was used as derivatizing agent to measure plasma concentrations of glyphosate and AMPA. The mean plasma concentration versus time data were sequentially fitted to 1, 2- and multiple-compartment models, using the computer program WinNonlin. The 2-compartment model was the best fit for both routes of exposure and was used to establish toxicokinetic characteristics. Plasma curves of glyphosate after single i.v. and oral administration and of

AMPA after single oral administration of glyphosate were fitted to equations. Absorption half-life, distribution rate constants for transfer of the test material from the central to the peripheral compartment and from peripheral to the central compartment, and the elimination rate constant were calculated by using the standard equations described by Wagner (1975, 1976). Area under the curve (AUC), total plasma clearance (CL), mean residence time (MRT), volume of distribution in the central and secondary compartments (V_1 and V_1, respectively), volume of distribution at steady state (V_{ss}), maximum plasma concentration (C_{max}), and time at which C_{max} was achieved (T_{max}) was determined by the study author.

4. Statistics – Mean plasma concentrations were calculated by the study author for use for 1-, 2- and multiple-compartment modeling, however, this data and the standard deviations were not presented. No other statistical analyses were performed.

II. RESULTS

A. Pharmacokinetic Studies

1. Absorption – After i.v. administration, a rapid distribution phase ($T_{1/2\alpha}$ = 0.35 hr) and a slower elimination phase ($T_{1/2\beta}$ = 9.99 hr) were observed. The volume of distribution at steady state (V_{ss}) was 2.99 L/kg and the plasma clearance (CL) value was 0.995 L/kg h.

After oral administration of glyphosate, the absorption was slow ($T_{1/2\ \alpha}$= 2.29 hr). Bioavailability of glyphosate after oral administration of 400 mg/kg bw was 23.21%. The maximum concentration of glyphosate (C_{max} = 4.62 µg/mL) was estimated 5.16 hour after oral administration. Glyphosate was distributed more slowly after oral than i.v. dosing (distribution half-lives, $T_{1/2\alpha}$ = 4.17 and 0.345 hr, respectively).

Table 2: Plasma kinetic parameters after administration of glyphosate

Group	Tmax (h)	C_{max} (µg/mL)	AUC(µg h/mL)	$T_{1/2\alpha}$ (h)	Clearance (L/kg h)	V_{ss} (L/kg^{-1})	Absolute bioavailability
Glyphosate							
100 mg/kg IV □	-	166.22	100.24	9.99	0.995	2.99	
400 mg/kg oral □	5.16	4.62	93.26	14.38	0.995	-	23.21%
AMPA							
400 mg/kg oral □	2.42	0.416	6.05	15.08	-	-	

[a] Data obtained from page 93 of the study report.

T_{max} Time to maximum plasma concentration.
C_{max} Maximum plasma concentration.
AUC Area under plasma concentration-time curve.
$T_{1/2}$ Half-life plasma elimination
V_{ss} Volume of distribution at steady-state

3. Tissue distribution – Tissue distribution studies were not conducted.

4. Excretion – Excretion was determined based on clearance of glyphosate or AMPA from the plasma. The excretion of glyphosate was slower after oral administration compared to i.v. dosing ($T_{1/2\alpha}$ = 14.38 hr vs. $T_{1/2\beta}$= 9.99 hr).

B. Metabolite characterization studies – AMPA was identified as the principal metabolite after oral administration of glyphosate to rats. The AUC for AMPA and AUC for glyphosate were used to calculate the fraction of glyphosate metabolized to AMPA. This fraction was calculated to be 6.49% of

the parent compound concentration in the plasma. Plasma concentration of AMPA (0.416 ug/ml) peaked at 2.42 hr after oral administration of glyphosate. The elimination half-life of AMPA was 15.08 hours.

III. DISCUSSION

A. Investigators' conclusions – "The present paper is the first to report in rats the plasma disposition of glyphosate using a selective HPLC analytical method to determine the levels of glyphosate and its metabolite AMPA in biological fluids in order to evaluate its pharmacokinetics. The validation parameters used show that the method is reliable and sensitive and allow an adequate characterization of the disposition of glyphosate in rats. In the study reported here, the kinetics of glyphosate after a single i.v. (100 mg/kg) and oral (400 mg/kg bw) administration were determined in rats as well as disposition of AMPA after oral administration of glyphosate were best described by use of two-compartment open model. Disappearance of glyphosate from plasma was characterized by an initial rapid distribution phase followed by a slower elimination phase.

After i.v. administration of 100 mg/kg, the distribution phase of glyphosate was fast ($T_{1/2}$ = 0.345 hr) and with a high value of volume of distribution at steady state (V_{ss} = 2.99 L/kg) which indicate that glyphosate is extensively distributed in extravascular tissues. The values of apparent volume of distribution in the second compartment (2.39 and 2.32 L/kg after i.v. and oral administration) also indicate that glyphosate easily penetrated all tissues, in agreement with data reported for Brewster et al. (1991). The elimination half-life calculated after i.v. administration was 9.99 h. The elimination half-life of glyphosate increased by 44% (to 14.38) after oral administration. This suggests that in rats the plasma disposition of glyphosate after oral administration is conditioned by the absorption process.

Glyphosate was slowly and poorly absorbed through the gastrointestinal tract in rats as reflected by an absorption half-life of 2.29 h, a maximal plasma concentration of 4.62 μg/ml and T_{max} of 5.16 hr after oral dose of 400 mg/kg. This T_{max} is comparable to previous studies using [^{14}C]-glyphosate, where glyphosate-derived radioactivity appeared to reach maximal tissue concentrations at 6.3 h after oral administration (Brewster et al. 1991). The oral bioavailability of glyphosate was 23.21% in rats, which was lower to those of other studies in which [^{14}C]-glyphosate administered at the oral dose of 10 mg/kg and approximately 30-36% of the dose was absorbed (Ridley and Mirley, 1988; Howe et al., 1988; Brewster et al. 1991). However, this result was close to the NTP study (NTP, 1992) which showed that approximately 19-23% of the administered 1000 mg/kg dose was absorbed as determined by urinary excretion data. Colvin and Millar (1973) also previously reported a poor oral absorption of ^{14}C-labelled glyphosate. When a single oral dose of glyphosate (6-9 mg/kg) was administered to New Zealand white rabbits, an 80% of the material appeared in the feces (Colvin and Millar, 1973). The low bioavailability of glyphosate may be caused by biliary excretion or glyphosate degradation at the site of absorption.

Glyphosate is poorly metabolized in rats. AMPA is the main metabolite in rats. The rate of elimination of AMPA ($T_{1/2}$ = 15.08h) after oral glyphosate administration was similar to that of glyphosate ($T_{1/2}$ = 14.38). The metabolite represented 6.49% of the parent drug plasma concentrations. A similar metabolic characterization was previously indicated by Brewster et al. (1991)."

B: Reviewer comments – This was a time-course toxicokinetics study in which the objective was to obtain the basic kinetic parameters of glyphosate and its metabolite AMPA in Wistar rats following oral and i.v. dosing. The kinetics of glyphosate after either oral or i.v. dosing was best described by a two-compartment model. The value and significance of these parameters are summarized below:

Absorption: The absorption half-life was calculated to be 2.29 hours while the maximal plasma concentration was determined to be 4.64 μg/ml and time to maximal plasma concentration was determined to be 5.16 hour after the oral administration of glyphosate. These parameters indicated a slow and poor absorption of glyphosate after oral administration

Distribution: The oral bioavailability fraction of glyphosate was 23.21% in rats. This result was close to

the NTP study (NTP, 1992) which showed that approximately 19-23% of the administered 1000 mg/kg dose was absorbed as determined by urinary excretion data. After i.v. administration of 100 mg/kg, the distribution phase of glyphosate was fast ($T_{1/2\alpha}$ = 0.345 hr) and with a high value of volume of distribution at steady state (V_{ss} = 2.99 L/kg) which indicated that glyphosate was extensively distributed in extravascular tissues. The values of apparent volume of distribution in the second compartment were 2.39 and 2.32 L/kg after i.v. and oral administration, respectively.

Metabolism: Glyphosate was poorly metabolized in rats. AMPA is the main metabolite which represented 6.49% of the parent plasma concentrations. The rate of elimination of AMPA ($T_{1/2}$ = 15.08 hr) after oral glyphosate administration was similar to that of glyphosate ($T_{1/2}$ = 14.38 hr).

Elimination: The elimination half-life of glyphosate after i.v. and oral administration was 9.99 h and 14.38 h, respectively. Oral administration of glyphosate resulted in 44% increase in the elimination half life compared to that of intravenous. After oral administration, the extended absorption of glyphosate delayed clearance from plasma relative to an IV dose.

This study is considered acceptable and classified as appropriate for qualitative use for hazard characterization of glyphosate. It is recognized that raw data was not provided in the study report; however, this report provided a relatively comprehensive understanding of the conditions under which the study was conducted and of the data generated. In addition, the effects noted in this study (e.g. bioavailability fraction, characteristics of ADME of glyphosate) are aligned throughout the glyphosate toxicology database (e.g. other metabolism studies show similar metabolism of glyphosate in rats).

C. Study deficiencies:

The following is a list of deficiencies according to OPPTS 870.7480; OECD 417 test guidelines.

1. Age of animals at the start of the study was not provided
2. Radiolabelled test substance using ^{14}C were not used for all components of this study including the metabolite identification. Although adequate toxicokinetic data are available on testing this compound using radiolabel ^{14}C, the analytical specificity and sensitivity of the method used (fluorescence detection) should be discussed in this paper compared to radiolabelled detection. In addition, the reason for choosing this method of detecting the compound in the plasma was not provided in the study report.
3. Two doses should have been used from ONE route of exposure since this information would aid in the dose response assessment of ADME characterization of the test substance. Although this was a time-course study in which the purpose was to obtain estimates of basic toxicokinetic parameters (e.g. C_{max}, T_{max}, half-life, AUC) for the test substance. The use of the second dose would have allowed characterization of substance bioavailability at different dose level or the effect of dose on clearance (e.g. to clarify whether clearance is saturated in a dose-dependent fashion).
4. When one dose level is used (per route of exposure), a rationale is required, as per the EPA and OECD test guidelines to explain why a second dose level was not included in the study design. Such rationale was not provided in the study report.
5. The study report did not specify whether a randomization process was used to select the animals for the two different exposure groups and during the time-course component of the study.

These deficiencies are not expected to impact the regulatory outcome of this study.

Reviewer # 1912 **Date** November 1, 2012

Study Type: Metabolism – Rat; OPPTS 870.7485 (Tier I & Tier II); OECD 417 (5.1.1/2).

Test Material (purity): Glyphosate (98.6% a.i.)

Synonyms: Glyphosate, technical grade (MON0573); Glycine, N-(phosphonomethyl); N-(phosphonomethyl)-glycine Round up® (MON 2139).

Citation: NTP Technical Report on Toxicity Studies of Glyphosate Administered in Dosed Feed to F344/N Rats and B6C3F1 Mice. Laboratory name: College of Pharmacy, University of Arizona, Tucson, AZ. Laboratory report number: Glyphosate, NTP Toxicity Report Number 16. NIH Publication 92-3135. Study report date: July 1992.

Sponsor: N/A

MRID: N/A

Executive Summary: In a metabolism study, ^{14}C-glyphosate (98.6% a.i.) was administered to 3-10 F344/N rats/group in single oral gavage doses of 5.6 or 56 mg/kg bw in distilled water,, in single intravenous or intraperitoneal doses of 5.6 mg/kg bw, or in a single oral dose of 5.6 mg/kg bw in animals pretreated with a 0.5 or 10 ppm dilution of Round Up® (MON 2139) in their drinking water.

A large percent of the administered dose (AD) was excreted in the feces following single low or high oral doses. Urinary excretion of the radioactivity was lower compared to the fecal excretion and accounted for a maximum of 35% of total administered radioactivity.

Peak blood radioactivity levels were reached within 1st and 2nd hours of oral administration for the low and high dose groups, respectively. The peak blood radioactivity level was about 0.20% of the AD for the low oral dose and about 0.70% of the AD for the high oral dose. The 10-fold increase in the oral dose resulted in a 35 fold increase in the peak blood concentrations. The blood radioactivity versus time plot fit a two-compartment model with a rapid distribution phase of 30 minutes and slower elimination phase of 13 hours. Blood radioactivity levels declined rapidly following an intravenous dose of 5.6 mg/kg such that within 6 hours of dosing, over 90% of radioactivity was recovered in the urine.

Most of the radioactivity levels in the tissues were recovered in the gastrointestinal (GI) tract (mostly in the small intestine) up to the 12 hour time point following single oral administration of the low and high doses. Radioactivity was also detected were liver, kidney, skin and blood, but in comparably small amounts compared to the small and large intestines (0.1-0.7% of AD in these tissues and at different time-points). Overall, the tissue radioactive residues decreased from 12% of total radioactivity to less than 1% within 24 hours. Comparison of the pattern of elimination following i.v. and oral administration of ^{14}C-glyphosate also supported the conclusion that the compound is incompletely absorbed.

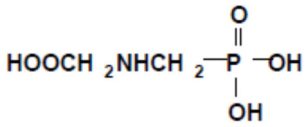
Although this study was not conducted according the guideline requirement for a metabolism study (OPPTS 870.7485; OECD 417), it is considered acceptable and classified as appropriate for qualitative use for hazard characterization of glyphosate.

Compliance: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were not provided. However, this study was peer-reviewed by NTP internally. The NTP peer-review panel

determined that the design and conditions of this study was appropriate and ensured that the toxicity study report presented the experimental results and conclusions thoroughly and clearly.

I. MATERIALS AND METHODS

A. Materials

Radiolabelled Test material:	^{14}C-glyphosate [N-(phosphono-^{14}C-methyl)-glycine]
Radiolabelled Purity:	99%
Test material	Glyphosate
Purity	98.6%
Structure:	
CAS #:	107-83-6
Stability:	3 weeks (stored in the dark, at room temperature)
Vehicle:	Deionized, distilled water
Positive control:	None
Test species:	Rat
Strain:	F344/N
Age at start:	Not provided
Weight at start:	170-280 g
Source:	Harlan-Sprague-Dawley (Indianapolis, IN)
Housing:	Individually in metabolic cages
Diet:	Wayne Lab Blox rat chow was provided *ad libitum*
Water:	Deionized water was provide ad libitum
Environmental conditions:	**Temperature:** Not provided **Humidity:** Not provided **Air changes:** Not provided **Photoperiod:** Not provided
Acclimation:	Not provided

B. Study Methods:

1. Study experimentation dates - Start: November 1987 End: November 1987

2. Group Arrangements - Animals were assigned to the test groups noted in Table 1. The study did not mention that the animals were randomly assigned to the test groups.

Table 1: Dosing groups for pharmacokinetic studies for glyphosate

Test Group	Dose (mg/kg bw)	Number	Time of Sacrifice Post-dose
Oral (Gavage)	5.6 mg/kg	3-10 ♂	3, 6,12, 24, 48, 72, 96hr
Oral (Gavage)	56 mg/kg	3-10 ♂	24, 48, 72hr
Intravenously	5.6 mg/kg	3-10 ♂	1,2, 3, 6, 12, 24 and 48 hr
Intraperitoneally	5.6 mg/kg	3-10 ♂	Not specified
Oral (gavage) - E	5.6 mg/kg	3-10 ♂	3, 6,12, 24, 48, 72, 96hr
Oral (gavage) - R	5.6 mg/kg	3-10 ♂	Day 16 after Roundup^{TM} exposure
Oral (gavage) - R	5.6 mg/kg	3-10 ♂	Day 1 before Roundup exposure

E = elimination studies

3. Dosing and sample collection – Male F344/N rats were fasted overnight prior to receiving a single gavage dose of ^{14}C-glyphosate in deionized, distilled water at levels of either 5.6 or 56 mg/kg bw. Urine and feces were collected at 24, 48, and 72 hours post treatment. One hundred µl of urine was mixed with 20 ml of Betaphase scintillation cocktail and analyzed for ^{14}C using a scintillation counter. Feces were weighed and mixed in 15 ml of 0.5 M NaOH for 24 hrs before homogenization. Aliquots of fecal homogenate were oxidized and then analyzed for ^{14}C.

At termination, aliquots of brain, heart, lung, liver, kidney, spleen, testes, muscle, skin, fat, small and large intestine, stomach, and blood were collected. The samples were weighed, oxidized, and analyzed for ^{14}C as described above; the contents of the small and large intestines and the stomach were analyzed separately for radioactivity. The resulting values were combined and added to the last fecal time point.

Additional groups of rats were given a single dose of glyphosate at a level of 5.6 mg/kg intravenously via the tail vein (dose volume 1.0 ml/kg), intraperitoneally, or orally to study the elimination of glyphosate. Urine and feces were collected analyzed for radioactivity over a 24 hour period.

Additional groups of rats were pretreated with Roundup® at 0.5 or 10 ppm in drinking water. For 16 days to determine the effect of the surfactants and inert ingredients on glyphosate absorption. The rats received a single oral dose of ^{14}C-glyphosate at 5.6 mg/kg, either on day one (prior to treatment with Roundup®) or on day 16 (when treatment with Roundup® was ceased).

Blood samples were obtained by cardiac puncture from rats given oral doses of glyphosate at 5.6 mg/kg or 56 mg/kg to determine the effect of dose on the absorption of glyphosate from the gastrointestinal (GI) tract. The samples were analyzed for radioactivity according to above described procedures.

4. Statistics – Statistical analyses were not conducted other than means and standard deviations.

II. RESULTS

1. Absorption and Excretion – A summary of percent radioactivity recovered in urine or feces is given in Table 2.

The peak blood radioactivity levels were reached at 1 and 2 hours for the 5.6 and 56 mg/kg oral dose groups, respectively. The peak radioactivity level reached up to 0.70% of the administered dose in the high dose group while this value was 0.20% of the administered dose in the low dose group (Figure 1 in the study report). Radioactivity levels in the blood rapidly declined following the intravenous dose of 5.6 mg/kg.

For both oral dose groups, about 35% of the radioactivity was recovered in the urine while 50-70% of radioactivity was recovered in the feces. At 72 hours, the 5.6mg/kg group had excreted 19% of AD in urine and 74% of AD in feces while the 56 mg/kg group had excreted 34% of the AD in urine and 58% of the AD in feces. These data (large percent of radioactivity recovered in the feces compared to urine) suggested a poor absorption in both low and high dose groups.

For the group exposed to the intravenous dose of 5.6 mg/kg, 90% of the radioactivity was eliminated through the urine in the first 6 hours indicating that excretion was fast and systemic exposure was minimal.

The study author stated that the apparent decrease in cumulative percentage eliminated in urine after the 5.6 mg/kg oral dose was probably due to inter-individual variation, and variances (from 10 to 3) in the number of animals per time point.

Data was not provided for the rats that were exposed to Roundup® (the isopropylamine salt of glyphosate and surfactants) in drinking water at concentrations of 0.5 to 10 ppm for 9 to 16 days. However, the study stated that no difference in elimination of an oral dose of 5.6 mg/kg of ^{14}C-glyphosate was noted following any of these exposures compared with the elimination of a similar dose a day prior to beginning administration with Roundup®.

Table 2: Cumulative Percentage of Oral or I.V. Dose of Glyphosate in Urine or Feces[a]

Time (hours)	Oral (5.6 mg/kg)		Oral (56 mg/kg)		I.V. (5.6 mg/kg)	
	Urine	Feces	Urine	Feces	Urine	Feces
6	10 ± 5	7 ± 11			90 ± 7	0.3 ± 0.2
12	31 ± 10	28 ± 10			95 ± 9	0.5 ± 0.5
24	26 ± 14	55 ± 13	28 ± 10	47 ± 12	98 ± 11	3 ± 2
48	18 ± 2	71 ± 8	33 ± 12	57 ± 15		
72	19 ± 2	74 ± 5	34 ± 12	58 ± 15		

[a] Data obtained from page 18 of the study report.

N = 3-10

3. <u>Tissue distribution</u> – The tissue distribution of radioactivity of the 5.6 mg/kg dose is summarized in Table 3.

Most of the radioactivity in the tissues was detected in the gastrointestinal tract (mostly the small intestine) up to the 12 hour time point. Radioactivity was also detected in liver, kidneys, skin, and blood to a much lesser extent when compared to the GI tract.

The blood radioactivity levels versus time plot fit into a 2-compartment model with an alpha (distribution) phase of about 0.5 hour and a beta (elimination) phase of 13 hours.

Table 3: Percent of Dose in Tissues Following Oral administration of glyphosate at 5.6 mg/kg[a]

Tissues	Time (hr)				
	3[b]	6[b]	12[b]	24[c]	96[c]
Small intestine	7.72 ± 1.74	10.20 ± 5.49	4.12 ± 2.25	0.48 ± 0.51	0.03 ± 0.01
Large Intestine	1.21 ± 1.07	0.51 ± 0.01	0.46 ± 0.28	0.17 ± 0.17	0.01 ± 0.00
Liver	0.10 ± 0.00	0.07 ± 0.04	0.11 ± 0.01	0.14 ± 0.08	0.05 ± 0.05
Kidney	0.36 ± 0.19	0.48 ± 0.42	0.31 ± 0.06	0.10 ± 0.07	ND
Skin	0.70 ± 0.45	0.18 ± 0.25	0.21 ± 0.12	ND[d]	ND
Blood	0.28 ± 0.01	0.18 ± 0.06	0.31 ± 0.10	0.03 ± 0.06	ND
Tissue Total	12.00 ± 0.33	11.67 ± 6.29	5.54 ± 2.35	0.89 ± 0.84	0.10 ± 0.06

[a] Data obtained from page 18 of the study report.

b N = 2 rats

c N = 3 rats

d ND notes that values were not determined as the amount of radioactivity in the samples below the level of accurate analytical measurement (<100dpm)

III. DISCUSSION

A. <u>Investigators' conclusions</u> – "Disposition studies showed that after a dose of glyphosate at either 5.6 or 56 mg/kg, over 70% of the administered dose was eliminated within 24 hours. Tissue distribution data indicate most of the radioactivity was in the gastrointestinal tract following oral administration, indicating the compound may not be completely absorbed. Comparison of the pattern of elimination following i.v.

and oral administration of [^{14}C]-glyphosate also supports the conclusion that the compound is incompletely absorbed. Radioactivity is eliminated primarily in feces after oral administration and primarily in urine following i.v. administration. If the usual assumption is made that i.v. administration represents the fate of a completely absorbed dose, the about 30% of the 5.6 mg/kg oral dose of glyphosate was absorbed; there is some evidence that a relatively higher percentage of the 56 mg/kg dose was absorbed. The 10-fold increase in dose resulted in a 30-fold increase in peak blood concentration. There also was a trend toward a higher percentage of the 56 mg/kg dose being eliminated in urine, but the differences were not statistically significant. Perhaps there is some interaction between glyphosate and the stomach/intestinal contents that binds a relatively larger percentage of the low dose, making it less available for absorption."

B: <u>Reviewer comments</u> – The objectives of this study were to obtain basic data on metabolism of glyphosate in F344/N rats following oral and i.v. dosing.

<u>Absorption</u>: The high percent (58-74% of AD) of radioactivity levels in the feces suggested a poor absorption of glyphosate following single low (5.6 mg/kg) or high (56 mg/kg) oral doses in rats. The peak blood radioactivity levels were reached within the first 2 hours of oral administration of the low or high dose. The peak radioactivity level reached up to 0.70% of the AD in the high dose group while this value was 0.20% of the AD in the low dose group, 2 and 1 hours post-dosing respectively. The internal concentration of the high dose would be 0.70% x 56 mg/kg = 0.392 mg/kg and the internal concentration of the low dose would be 0.20% x 5.6 mg/kg = 0.0112 mg/kg. So a 10-fold increase in dose resulted in a 35-fold (0.392 mg/kg / 0.0112 mg/kg) increase in peak blood concentration (not 30-fold as the study author stated in the report). A ratio of 3.5:1 increase in blood concentrations of a compound compared to oral doses would indicate saturation of excretory pathways as the organisms could not eliminate the test compound as fast as the low dose.

<u>Distribution</u>: The highest radioactive tissue residues were detected in the GI tract. This finding was consistent with a large fecal excretion of the administered radioactivity and the study report did not discern whether the radioactive residues were in this tissue or associated with the fecal matter that was still retained in the intestines. Other organs where radioactivity was detected were liver, kidney, skin, and blood. The study reported (although the data was not shown) that the blood radioactivity versus the time plot fit a two-compartment model with a rapid distribution phase of 30 minutes and slower elimination phase of 13 hours.

<u>Elimination</u>: Following oral administration of ^{14}C-glyphosate, elimination was similar in the low and high dose groups although a higher percentage (58-74%) of radioactivity excreted through the feces and a lower portion (~ 35%) excreted through the urine. The fecal excretion peaked towards the end of the measurement (72 hour time point) for both dose groups. The urinary excretion of the radioactivity plateaued at 12 hours in the low dose group and at 72 hours in the high dose groups. Following the intravenous administration of a low dose (5.6 mg/kg) of ^{14}C-glyphosate, the elimination was rapid (90% excreted within 6 hours) and occurred primarily through the urine.

This study is considered acceptable and classified as appropriate for <u>qualitative</u> use for hazard characterization of glyphosate. It is recognized that raw data was not provided in the study report; however, this report provided a sufficient understanding of the conditions under which the study was conducted and of the data generated. In addition, the effects noted in this study (e.g. characteristics of ADME of glyphosate) are aligned with other lines of evidence throughout the glyphosate toxicology database (e.g. other metabolism studies show similar metabolic profile for glyphosate in rats).

C. <u>Study deficiencies</u>:

The following is a list of deficiencies according to OPPTS 870.7480; OECD 417 test guidelines.

1. The exact number of rats per group or per time point used was not specified.
2. Environmental conditions (such as temperature, humidity levels, etc) were not provided for this study.
3. Age of animals at the start of the study was not provided
4. Acclimation period for the animals was not provided.
5. The study report did not state whether animals were randomly assigned to the test groups.
6. The data/results of group of animals that were exposed to glyphosate intraperitoneally were not discussed in the study report although discussed as part of design/materials and methods.
7. The data/results for tissue distribution in the high dose oral gavage group were not presented.

These deficiencies are not expected to impact the regulatory interpretation of this study.

Note To/Note au: Toxicology File

From/De: Haris Gisavi
Evaluation Officer, TRS-2, HED

Subject/Objet: **Sub. No(s):** 2004-0175
Product Name: Glyphosate products
Active Ingredient: Glyphosate acid and salt forms (GPS)

Action Requested : **Screen/Review of Open Literature Studies**

Study Title: **Glyphosate impairs male offspring reproductive development by disrupting gonadotropin expression.**

Summary: In this study, glyphosate formulation (commercially available, called Roundup Transorb) was administered to 12 pregnant female Wistar rats at a dose level of 50 mg/kg bw from GD 18 to PND5. On PND60, the male offspring from these females were assessed for various reproduction parameters. Theses parameters included sexual partner preference, sexual behaviour, hormone measurements, mRNA expression of hormones, protein expression of the hormones, sperm evaluation, histology and morphometry of seminiferous epithelium, organ weights, and body weight (also tested on PND21, PND 30, PND40). The males from treated dams spent more time in contact with females than control animals. An increase in the latency to first, latency to first intromission and latency to mount after first ejaculation was noted in treated males compared to controls. Statistically significant increased testosterone and estradiol serum concentrations were noted in the treated animals compared to controls. Increased sperm production was noted in the treated animals compared to controls. An increase in epithelial height and a reduction in luminal diameter without changes in the tubular diameter were noted in the treated animals compared to controls.

Limitations:

- The test substance was a glyphosate based commercial formulation. The percent active ingredient was not indicated. Other components of the formulation were not identified besides the glyphosate and isopropylamine salt of glyphosate. The remaining components were identified as 'inert' ingredients.

- One dose group was used compared to at least three dose groups that are generally used in the guideline studies.

Conclusion: This study does not meet the minimum criteria to be eligible for risk assessment of the active ingredient, as outlined in the draft "Guidance for considering and using open literature studies to support human health risk assessment" (US EPA, 2011) and the PMRA document (PMRA# 2158384). The major limitations as discussed above were not using the active ingredient as the test substance and inadequate number of dose levels. This study is categorized as 'invalid' (inappropriate for quantitative or qualitative use in risk assessment because it is of insufficient quality and lacks scientific defensibility).

Reference: Romano, M.A. (2012). Glyphosate impairs male offspring reproductive development by disrupting gonadotropin expression. Arch. Toxicol. 86:663-673

Study Title: **Vitamin C and E reverse effect of herbicide-induced toxicity on human epidermal cells HaCaT: a biochemometric approach**

Summary: In this *in vitro* study, human keratinocytes cell line HaCaT was used to study cytotoxicity of glyphosate alone or included in Roundup 3 plus® formulation and the effects of Vitamin C and Vitamin E in the protection against cytotoxicity (if any). The cell cultures were incubated overnight in increasing concentrations of glyphosate or Roundup (0-25mM) and/or Vitamin E or C. Various combinations of glyphosate or roundup with various concentrations of Vitamin E and/or C were tested in different incubation periods. Cytotoxicity was measured thereafter and the inhibition concentration 50% (IC_{50}) was determined. The preliminary IC_{50} values of glyphosate alone and roundup on the cell cultures tested were 22mM and 19.5 mM. According to this result, the authors stated that the formulation is more toxic compared to the active ingredient. Optimal values for vitamin C and vitamin E were calculated from the results of this study that were needed to modulate the toxic effect of the glyphosate formulation. The study authors concluded that glyphosate based formulations can be responsible for oxidative damage to human epidermal cells and antioxidant compounds can decrease this effect.

Limitations:

- The percent active ingredient (glyphosate) was not indicated. The components comprising the glyphosate formulation used as the test substance were not provided.

- Inadequate description of the data in the results section as the IC_{50} values could not be obtained for glyphosate alone (the active ingredient) before the addition of Vitamin C or E.

- The challenge of extrapolating from the results of this (*in vitro*) study to the complex biology of whole animals.

Conclusion: This study does not meet the minimum criteria to be eligible for risk assessment of the active ingredient, as outlined in the draft "Guidance for considering and using open literature studies to support human health risk assessment" (US EPA, 2011) and the PMRA document (PMRA# 2158384). The major limitation as discussed above was not providing the purity level of the active ingredient. This study is categorized as 'invalid'.

Reference: Nicod, Laurence, et al. (2005). Vitamins C and E reverse effect of herbicide-induced toxicity on human epidermal cells HaCaT: a biochemometric approach. International Journal of Pharmaceutics 288: 219-226

Study Title: **Glyphosate-based pesticides affect cell cycle regulation**

Summary: In this *in vitro* study, fertilized sea urchin eggs and the embryos were incubated with fresh seawater (control) or five different glyphosate formulations. The results showed that the formulations impeded the cell division process in a dose dependent manner, ranging from a delay in the time of the cell division up to an inhibition of the process. The effects of formulations on cell cycle regulation were compared with their glyphosate content. The study authors calculated a threshold adverse dose of glyphosate sufficient to provoke dysfunction of at least one cell was equal to 10 μM when present in the formulations tested. However, the purity of glyphosate in these formulations was not given. The study authors stated that these formulations contain glyphosate at a concentration of 40mM which is 500 to 4000 times higher concentration that the threshold adverse concentration towards the cell cycle estimated by the data of this study.

Limitations:

- The test substances were glyphosate based commercial formulation. The percent active ingredient was not indicated. Other components of the formulation were not identified besides glyphosate.

- The challenge of extrapolating from the results of this (*in vitro*) study to the complex biology of whole animals.

Conclusion: This study does not meet the minimum criteria to be eligible for risk assessment of the active ingredient, as outlined in the *draft* "Guidance for considering and using open literature studies to support human health risk assessment" (US EPA, 2011) and the PMRA document (PMRA# 2158384). The major limitation as discussed above was not using the active ingredient as the test material. This study is categorized as 'invalid'.

Reference: Marc, Julie, et al (2004). Glyphosate-based pesticides affect cell cycle regulation. Biology of the Cell 96: 245-249.

Study Title: **A glyphosate-based herbicide induces necrosis and apoptosis in mature rat testicular cells *in vitro*, and testosterone decrease at lower levels.**

Summary: In this *in vitro* study, glyphosate and Roundup were tested on male rat testicular cells from 1 to 10000 ppm. Roundup and glyphosate caused a reduction in testosterone by 35% in the Leydig cells at 1ppm, but at concentrations higher, the difference between control and treated cells were comparable. At concentrations 75 and 100 ppm, the testosterone levels were increased compared to controls. Roundup resulted in necrosis and apoptosis in Leydig cells, germ cells and Sertoli /germ cells co-cultures starting from 0.1% dilution (1000 ppm). Glyphosate resulted in necrosis of Sertoli and germ cells mixtures and in isolated germ cells starting at 5000 ppm.

Limitations:

- Purity of active ingredient was not provided in the paper
- The components of the formulation were not characterized in this study
- The challenge of extrapolating from the results of this (*in vitro*) study to the complex biology of whole animals.
- The study report did not discuss whether the tested concentrations of glyphosate would produce cytotoxicity and impact the results of this study

Conclusion: This study showed that the glyphosate formulation (roundup) or glyphosate (at unknown purity level) increased adenylate kinase activity (a biomarker of cell damage or necrosis) in the culture media. This study is considered 'invalid' or of little utility for hazard assessment of glyphosate because of limitations noted above.

Flags: Glyphosate resulting in necrosis of Sertoli cells and germ cells starting at 5000 ppm (at unknown purity level).

Reference: Chair, E. et al. (2012). Glyphosate-based herbicide induces necrosis and apoptosis in mature rat testicular cells in vitro, and testosterone decrease at lower levels. Toxicology *in Vitro* 26: 269-279.

Study Title: **Placental Passage of Benzoic Acid, Caffeine, and Glyphosate in an *ex vivo* Human Perfusion System.**

Summary: In this *ex vivo* study, placentas from uncomplicated pregnancies were used as perfusion models in which labelled and unlabelled glyphosate were added and samples were collected before and after perfusion. The transfer of glyphosate was restricted throughout perfusion, with a lower permeation rate which was speculated to be due to its hydrophilicity. Overall about 15% glyphosate in maternal circulation crossed to the fetal circulation during the study period and in the seven perfusion models tested.

Limitations:

- Purity of glyphosate was not given
- The challenge of extrapolating from the results of this (*ex vivo*) study to the complex biology of whole animals.

Conclusions: This study showed that in the *ex vivo* environment, glyphosate could cross a placental membrane and reach fetal circulation at a lower fraction of the concentration available at maternal circulation. However, this study is considered 'invalid' or of little utility for hazard assessment of glyphosate because of limitations noted above.

Flag: Around 15% of the glyphosate in maternal circulation crossed to the fetal circulation in perfusion models tested in this study

Reference: Mose, Tina *et al*. (2008). Placental passage of Benzoic acid, Caffeine, and Glyphosate in an ex vivo human perfusion system. Journal of Toxicology and Environmental Health, Part A, 71: 984:991.

Study Title: **Alteration of estrogen-regulated gene expression in human cells induced by agricultural and horticultural herbicide glyphosate.**

Summary: In this *in vitro* study, DNA microarray analysis was used to study the effects of glyphosate to alter the expression of a variety of genes in human cells. Real time PCR was used to corroborate the altered states of expression of these genes. Three genes HIF1, CXCL12 and EGR1 as determined by DNA microarray analysis and quantitative real time PCR were dysregulated by glyphosate exposure. The potential gene regulation effects included initiation of apoptosis in cells of cerebral and myocardial tissues, increase angiogenesis and other possible effects listed in the study report. Overall, the exposure of glyphosate combined with estrogen resulted in increased dysregulation of the three genes (HIF1, CXCL12 and EGR1) compared to glyphosate or estrogen alone.

Limitations:

- The cell lines used were not adequately characterized.
- The challenge of extrapolating from the results of this (*in vitro*) study to the complex biology of whole animals.
- The purity of glyphosate was not provided.
- The concentration of glyphosate used was ambiguous and difficult to interpret and convert to part per million or mg/kg bw.

Conclusions: This study showed that the glyphosate alone or with estrogen increased dysregulation of the selected genes. However, this study is considered 'invalid' or of little utility for hazard assessment of glyphosate because of limitations noted above.

Flags: Glyphosate resulted in dysregulation of genes mainly responsible for initiation of apoptosis in various tissue types.

Reference: Hokanson, R, et al. (2007). Alteration of estrogen-regulated gene expression in human cells induced by the agricultural and horticultural herbicide glyphosate. Human & Experimental Toxicology. 26: 747-752.

Note To/Note au: Toxicology File

From/De: 2032
Evaluation Officer, TRS-2, HED

Subject/Objet: **Sub. No(s):** 2004-0175
Product Name: Glyphosate products
Active Ingredient: Glyphosate acid and salt forms (GPS)

Action Requested : **Screen of Open Literature Studies**

Study Title: The oral and intra-tracheal toxicities of Roundup and its components to rats.

Summary: Toxicity of Roundup and its components, glyphosate and polyoxyethyleneamnine (POEA) was examined in Wistar rats after intra-tracheal and oral administration. Immediate respiratory effects were more severe and longer lasting in animals exposed to treatments containing POEA. Two animals treated orally with POEA died within 24 hours. Mortality was noted across all treatment groups after intrat-racheal treatment, with lowest mortality rate noted in the glyphosate treated group. Glyphosate treatment produced transient diarrhea, mild lung hemorrhages, and lung epithelial cell damage, primarily post-intratracheal exposure. The results indicated that POEA containing preparations and POEA treatment were more toxic than glyphosate treatment in Wistar rats.

Methods
Five groups of fasted Wistar rats (250-350g), 8/group, were anesthetized with sodium pentobarbitone (50 mg/kg ip) and atropine sulphate (50 mg/kg im) and administered intratracheally glyphosate (200 mg/kg), POEA (100 mg/kg), mixture of glyphosate (200 mg/kg) and POEA (100mg/kg), Roundup (equivalent to 200 mg/kg of glyphosate and 100 mg/kg of POEA as 41% w/v glyphosate isopropylamine salt and 18% w/v POEA) or saline (control animals) at 1mL/kg bw. Another five groups of 8 rats/group were treated orally, via gavage, with 2000 mg glyphosate/kg , 1000 mg POEA/kg, a mixture of 2000 mg glyphosate/kg and 1000 mg POEA/kg, Roundup (2000 mg glyphosate/kg and 1000 mg POEA/kg), or saline at 10mL/kg. Animals were observed at 0, 1, 3, 6 and 24 hours after dosing, and then sacrificed via halothane overdose for necropsy. Lung tissues were weighed, graded (normal, mild, moderate or severe) for hemorrhage and fixed in formalin for histologic examination. Tissues were stained with hematoxylin and eosin and were analysed for presence of hemorrhage and epithelial cell damage and/or loss with a severity grading. Statistical analysis was performed, using Fisher exact test for comparison between the groups (clinical signs and lung hemorrhage), and Anova with an LSD test for ratios and means.

Results
Within the first hour of intratracheal administration of Roundup or its components, death was observed, with 50% animals dying in Glyphosate+POEA group, and 37.5% animals dying in Roundup and POEA groups, as noted in Table 1. Nose bleeds, loud breathing, wheezing and gasping were observed in rats exposed Roundup or its constituents, but the observations were transient, with the number of animals affected decreasing with time, and no symptoms of toxicity visible by 24 hours post-dosing. Glyphosate intratracheal administration, as compared to POEA administration, caused less toxic symptoms.

Examination of lung hemorrhaging from intratracheally dosed animals, showed 75% of lungs affected in the glyphosate+POEA group, however the hemorrhaging was mild (Table 2). Roundup and POEA groups had both 12.5% of moderate hemorrhaging visible.

Animals that received Roundup or glyphosate+POEA orally had comparable reactions to intratracheal administration. However, animals given POEA orally were the only ones that showed mortality and had highest incidence of nose bleeds and diarrhea, as compared to other treatment groups. Mortality was noted after 24 hours in the rats given POEA orally. Prior to death, the animals in that group were noted as having the highest incidence of diarrhea and nose bleeds that increased with time post-dosing.

Animals treated with POEA orally showed the greatest range of hemorrhaging, with 50% of lung affected, including 12.5% of moderate grade and 12.5% of severe grade. Lung wet weight/body weight in the POEA group had large standard deviation; however, there was no raw data available for further examination.

Table 1. Signs of toxicity (incidence after 0, 1, 3, 6 and 24 hours per 8-dead animals) after intratracheal and oral administration of Roundup and its constituents

Intratracheal Administration Results					
Symptoms	Saline (n=8)	Roundup (n=8, 5)	Glyphosate+POEA (n=8, 4)	Glyphosate (n=8, 6)	POEA (n=8, 5)
Cumulative death	0	0, **3, 3, 3, 3**	0, **4, 4, 4, 4**	0, **2, 2, 2, 2**	0, **3, 3, 3, 3**
Nose bleed	0	8, 2, 2, 0, 0	7, 2, 2, 0, 0	0, 2, 0, 0, 0	4, 3, 1, 0, 0
Loud breathing	0	5, 2, 2, 0, 0	5, 2, 2, 0, 0	1, 1, 0, 0, 0	4, 1, 1,0, 0
Wheezing	0	4, 2, 2, 0, 0	4, 2, 1, 0, 0	1, 1, 0, 0, 0	3, 1, 1, 0, 0
Gasping	0	7, 2, 2, 0, 0	6, 2, 2, 0, 0	3, 2, 0, 0, 0	5, 2, 1, 0, 0
Oral Administration Results					
Symptoms	Saline (n=8)	Roundup (n=8)	Glyphosate+POEA (n=8)	Glyphosate (n=8)	POEA (n=8, 6)
Cumulative death	0	0	0	0	0, 0, 0, 0, **2**
Nose bleed	0	0, 0, 0, 1, 1	0, 0, 0, 0, 1	0	0, 0, 0, 0, 3
diarrhea	0	0, 0, 0, 5, 7	0, 0, 2, 5, 8	0,0, 0, 2, 0	0, 1, 4, 7, 6

Table 2. Gross Lung Hemorrhage (%) results after administration of Roundup and its components

Lung Hemorrhage after intratracheal administration					
Symptoms	Saline (n=8)	Roundup (n=8)	Glyphosate+POEA (n=8)	Glyphosate (n=8)	POEA (n=8)
Normal	87.5	37.5	25	75	50
Mild	12.5	50	75	25	37.5
Moderate	0	12.5	0	0	12.5
Severe	0	0	0	0	0
Total	12.5	62.5	75	25	50
Lung wet wt/body (10^{-3})	6.37±0.76	7.10±1.22	8.48±2.18	6.40±0.99	7.39±2.05
Lung Hemorrhage after oral administration results					

Symptoms	Saline (n=8)	Roundup (n=8)	Glyphosate+POEA (n=8)	Glyphosate (n=8)	POEA (n=8)
Normal	100	62.5	50	87.5	50
Mild	0	37.5	50	12.5	25
Moderate	0	0	0	0	12.5
Severe	0	0	0	0	12.5
Total	0	37.5	50	12.5	50
Lung wet wt/body wt (10^{-3})	5.99±1.19	5.79±0.78	6.39±0.94	6.28±0.69	7.65±3.19

Gross observation of lung hemorrhage was supported by histological data. Histopathological analysis indicated that rats exposed to POEA had overall more animals with severe hemorrhage and epithelial loss/damage, as compared to other groups, by both routes of administration.

Table 3. Histological changes in lungs from rats exposed to Roundup and its components

Intratracheal administration					
Symptoms	Saline (n=6)	Roundup (n=5)	Glyphosate+POEA (n=6)	Glyphosate (n=5)	POEA (n=4)
Normal hemorrhage	6	3	6	5	1
Severe hemorrhage	0	2	0	0	3
Normal epithelial cell loss/damage	6	3	5	3	3
Severe epithelial cell loss/damage	0	2	1	2	1
Oral administration results					
Symptoms	Saline (n=6)	Roundup (n=6)	Glyphosate+POEA (n=5)	Glyphosate (n=6)	POEA (n=6)
Normal hemorrhage	6	6	5	5	4
Severe hemorrhage	0	0	1	1	2
Normal epithelial cell loss/damage	6	5	5	6	4
Severe epithelial cell loss/damage	0	1	1	0	2

Discussion: The toxicity was almost immediate after intratracheal administration of test substances, as compared to oral, even with 10% of the doses used. Combination of glyphosate+POEA was chosen as one of treatment groups, with ratio of 2:1 to simulate the concentrations found in Roundup, which was also chosen as one of the treatment groups. The results between these groups were similar. Examination of results showed POEA treatment to cause the most toxicity, with the greatest mortality rate. Nose bleeds were seen only with POEA-containing oral preparations.
Data indicated that glyphosate and POEA can cause lung damage. Comparison of toxicities of glyphosate, POEA and Roundup given by oral and intratracheal route to Wistar rats showed that the surfactant POEA was more toxic than the glyphosate alone, and glyphosate+POEA and Roundup toxicity was greater than toxicity of glyphosate alone. There was no synergistic increase in the toxicity of the active ingredient of the herbicide and the surfactant.

Limitations:

- The purity of the chemicals was not specified The study included not only EP but also the components, the a.i. and surfactant but without multiple dose levels (no dose response, and therefore not possible to choose a NOAEL/LOAEL).

- The n value was 8/group, reducing the strength of statistical analysis

- The range of body weights was larger than expected (difference of 100g). at the point of assignment to treatment groups.

- Study did not include body weight, body weight gain and food consumption data

Conclusion: This study does not meet the minimum criteria to be eligible for risk assessment of the active ingredient, as outlined in the draft "Guidance for considering and using open literature studies to support human health risk assessment" (US EPA, 2011). The major limitations as discussed above were insufficient number of both dose levels and animals. This study is categorized as 'invalid' (inappropriate for quantitative or qualitative use in risk assessment because it is of insufficient quality and lacks scientific defensibility). However this study provides useful information about effects of the surfactant.

Reference: Adam, A., Marzuki, A/, Abdul Rahman .H. and Abdul Aziz M. (1997). The oral and intratracheal toxicities of Roundup and its components to rats. Vet human Toxicol. 39: 147-51.

Oct 15, 2012

Note To/Note au: Toxicology File

From/De: 2032
Evaluation Officer, TRS-2, HED

Subject/Objet: **Sub. No(s):** 2004-0175
Product Name: Glyphosate products
Active Ingredient: Glyphosate acid and salt forms (GPS)

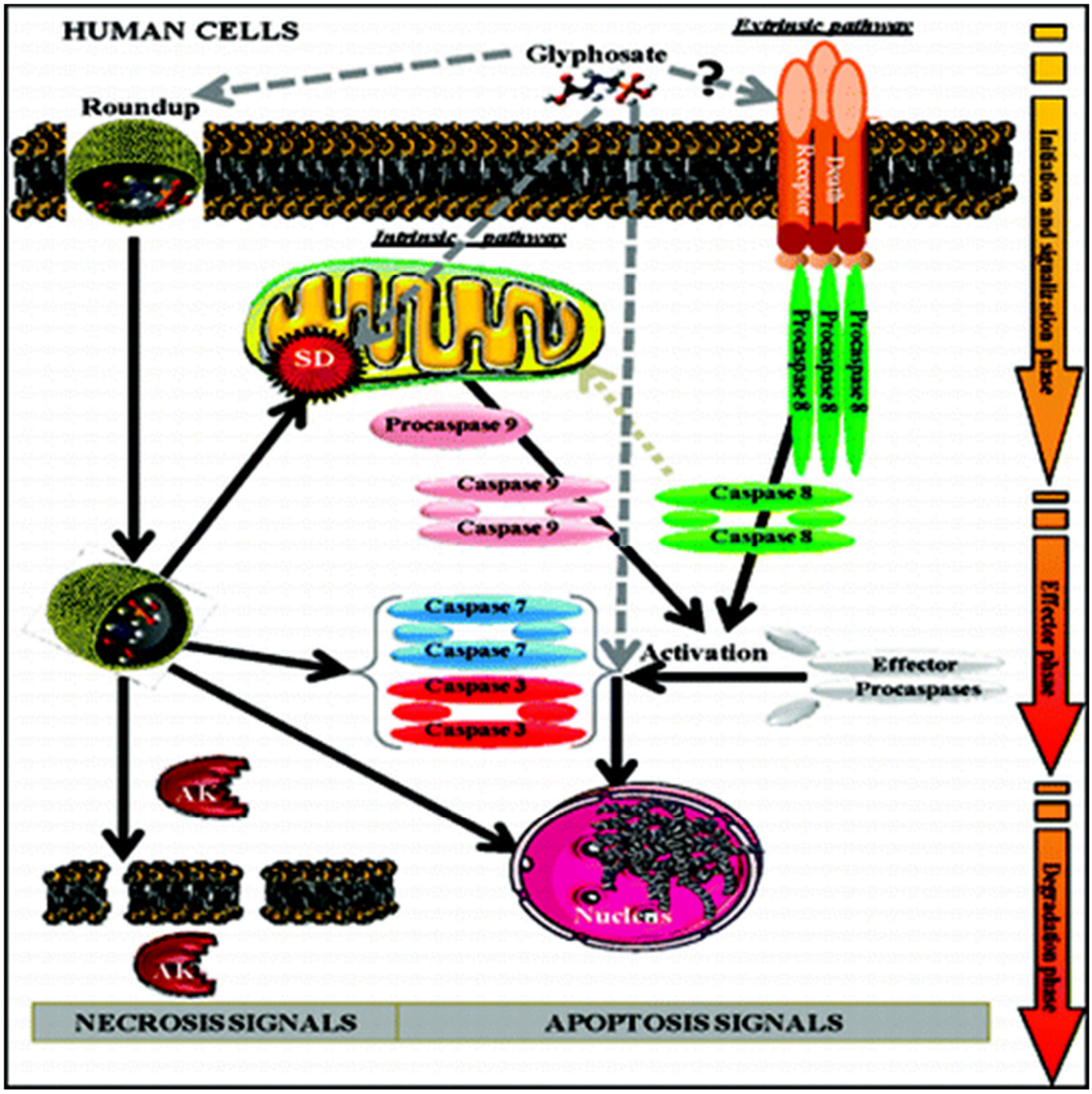
Action Requested : **Screen of Open Literature Studies**

Study Title: Glyphosate Formulations Induce Apoptosis and Necrosis in Human Umbilical, Embryonic, and Placental Cells.

Summary: The toxicity of four glyphosate based herbicides in Roundup formulations was tested using three different human cell types, and compared to results obtained with glyphosate, its main metabolite AMPA or with surfactant POEA. The dilutions used were far below agricultural recommendations and corresponded to low levels that might be found as residues in food or feed. The cell lines included HUVEC primary neonate umbilical cord vein cells, 293 embryonic kidney and JEG3 placental cell lines.

Four Roundup herbicide formulations (Roundup Express 7.2 g/L of glyphosate, Bioforce or Extra 360 with 360 g/L of glyphosate, Grands Travaux with 400 g/L of glyphosate, and Grands Travaux plus with 450 g/L of glyphosate) were evaluated for cytotoxic effects in the three cell lines. Mitochondrial succinate dehydrogenase (SD) activity (cell aspyxia as shown by enzyme inhibition) and release of cytoplasmic adenylate kinase (AK activity indicative of membrane damage/rupture during necrosis and/or a secondary necrosis at the end of apoptosis) were measured after 24 hours of exposure to evaluate cytotoxicity. AK measurements were 1.5-2 more sensitive than SD measurements. All formulations caused total cell death within 24 h, but unexpectedly, Grands Traveaus 400 formulation was the most toxic, and reached LD50 in all the cell lines at the lowest dilution of ~ 0.01 corresponding to 47µM of glyphosate (8ppm with adjuvants). The next most toxic treatment was with Grands Traveaus 450 which has higher glyphosate content (~0.1 dilution), the Bioforce, Roundup Express (lower glyphosate content, undiluted) and glyphosate itself. Without the adjuvants, 4-10 ppm of glyphosate did not show similar toxicity.

Formulations including AMPA and POEA provoked SD and AK effects in human cell lines at different concentrations, with POEA being the most potent (altering SD in HUVEC at 1ppm and AK in 293 and JEG3 cell lines), followed by Roundup formulations, and then glyphosate and AMPA. AMPA was more toxic than glyphosate on human cells, since the metabolite can destroy cell membrane and provoke AK release (more sensitive damage); however glyphosate was 3-8 times more inhibitory to SD than AMPA.



When compounds were tested in pairs (glyphosate+POEA, glyphosate+AMPA or AMPA+POEA) to determine combined effects on cell membrane integrity via AK release, the mixtures were more disrupting in combination when used on embryonic and umbilical cells.

Roundup 360 and glyphosate were shown to also induce apoptosis via activation of enzymatic caspases 3/7 activity from 6 hours with a maximum at 12 hours, with activation being 60-160 more times sensitive in HUVEC cells than the other cell lines. Glyphosate activated the caspases at a lower concentration than that which inhibited SD activity. After 24 hours of treatment, the caspases 3/7 activity returned to basal levels, showing expected gradual loss of caspases 3/7 activity usually noted in apoptotic cells that undergo secondary necrosis *in vitro*. Low concentration of glyphosate formulations caused a change in cell morphology with the cell death, as indicated by lack of adhesion, shrinkage and fragmentation in apoptotic bodies, confirmed with the DNA fluorescent labeling with DAPI (DNA condensation).

In conclusion, the glyphosate formulations' adjuvants like POEA are not "inert", but change human cell permeability and amplify toxicity induced already by glyphosate (apoptosis), disrupting cell and mitochondrial membranes and promoting apoptosis and necrosis.

Limitations:

- The study included glyphosate formulations, in addition to glyphosate itself, surfactant and primary metabolite AMPA; however the purities of chemicals were not specified.

- Data was shown only in a graph format, without access to raw data tables

- The *in vitro* effects in a closed system are difficult to translate into *in vivo* effects where metabolism and clearance would play a large role in potential toxicity

Conclusion: This study does not meet the minimum criteria to be eligible for risk assessment of the active ingredient, as outlined in the draft "Guidance for considering and using open literature studies to support human health risk assessment" (US EPA, 2011). The major limitation as discussed above was incomplete characterization of the test substances, and unknown relevance of *in vitro* effects to *in vivo* effects. This study is categorized as 'invalid' (inappropriate for quantitative or qualitative use in risk assessment because it is of insufficient quality and lacks scientific defensibility).

Flag: Glyphosate caused apoptosis in umbilical (most sensitive), embryonic and placental cell lines *in vitro*, activating caspases 3/7 at concentrations that did not provoke cell and mitochondrial membrane damage.

Reference: Nora Benachour and Gilles-Eric Séralini (2009). Glyphosate Formulations Induce Apoptosis and Necrosis in Human Umbilical, Embryonic, and Placental Cells. Chem. Res. 22 (1), pp 97–105.

Note To/Note au: Toxicology File

From/De: 2032
Evaluation Officer, TRS-2, HED

Subject/Objet: **Sub. No(s):** 2004-0175
Product Name: Glyphosate products
Active Ingredient: Glyphosate acid and salt forms (GPS)

Action Requested : **Screen of Open Literature Studies**

Study Title: Roundup revelation: weed killer adjuvants may boost toxicity.

Summary: This was a commentary on a journal article investigating Roundup toxicity and glyphosate alone on cultured placental cells. The formulation was found to kill the cells at concentrations far below those used in agricultural practice, and was found to be twice as toxic as glyphosate alone. Further research found that Roundup disrupted aromatase activity at concentrations 100 times lower than those used in agriculture. However it is unknown how the results from the *in vitro* experiments translate to effects in the whole body.

Limitations:

- Commentary on a study only, incomplete data

Conclusion: This study does not meet the minimum criteria to be eligible for risk assessment of the active ingredient, as outlined in the draft "Guidance for considering and using open literature studies to support human health risk assessment" (US EPA, 2011). The major limitation as discussed above was based on deficiency in reporting. This study is categorized as 'invalid' (inappropriate for quantitative or qualitative use in risk assessment because it is of insufficient quality and lacks scientific defensibility).

Reference: Bonn, D. (2005). Roundup revelation: weed killer adjuvants may boost toxicity. International health Perspectives 13(6): A403-404.

Note To/Note au: Toxicology File

From/De: 2032
Evaluation Officer, TRS-2, HED

Subject/Objet: **Sub. No(s):** 2004-0175
Product Name: Glyphosate products
Active Ingredient: Glyphosate acid and salt forms (GPS)

Action Requested : Screen of Open Literature Studies

Study Title: Pre- and postnatal toxicity of the commercial glyphosate formulation in Wistar rats.

Summary: Sexually mature Wistar rats, 15 animals/sex/group, were treated by gavage (10 ml/kg) with Roundup® formulation (Monsato of Brazil; containing 360g/l of glyphosate and 18% polyoxyethyleneamine surfactant), at 0, 50, 150 or 450 mg/kg bw during pregnancy (21-23 days) and lactation (21 days).

Maternal toxicity was not observed. Treatment did not affect body weight nor body weight gain in the animals. The offspring variables, including litter size, number of live and dead pups, and viable pups, were unaffected. The authors noted that sex ratio of male to female pups was unaffected, however there seems to be a statistically non-significant decrease in the number of females at the high dose (85/160, 81/161, 82/165 and 69/162). Preputial separation was shortened by about a day at the high dose, and vaginal canal opening was delayed by two days across the dose groups, showing statistical significance but no dose response. The authors used historical control data from within their department to validate the sexual maturation results; however the historical control data was not available in the article.

Reproductive effects were noted in male offspring, in absence of maternal toxicity. The percentage of abnormal sperm was increased at puberty, but no clear dose-response was noted. Testosterone concentration decreased with increasing dose, and showed statistical significance at puberty (-23%, -38% and -71%, as compared to control even though preputial separation was attained earlier), but not in adulthood. Treated adult males also showed decreased sperm production and sperm number/epididymis tail (statistically significant at low and high dose) but without a dose response. In addition, high dose adult males showed decreased (~-29%) tubules with spermatogenesis, as compared to control. In addition, growth disorder and degeneration was observed at puberty in the two highest dose groups (4/5 and 4/5 testis), characterized by a decrease in elongated spermatid and the presence of vacuolization. Degeneration, as characterized by the absence of tubular lumen for all doses (3/5, 4/5 and 5/5 testis) was also noted in all treated adult males.

The noted toxicity in the animals cannot be attributed to the active ingredient alone, since a glyphosate formulation was used.

Limitations:

- The test substance was a glyphosate commercial formulation

- The percentages of active ingredient were indicated but purity of glyphosate was not specified. Other components of the formulation were not identified.

- Only 5 animals per dose were observed at microscopic level.

Conclusion: This study does not meet the minimum criteria to be eligible for risk assessment of the active ingredient, as outlined in the draft "Guidance for considering and using open literature studies to support human health risk assessment" (US EPA, 2011). The major limitations as discussed above were not characterizing the test substance properly, and experiments focusing on formulation not the active ingredient itself. This study is categorized as 'invalid' (inappropriate for quantitative or qualitative use in risk assessment because it is of insufficient quality and lacks scientific defensibility).

Reference: Eliane Dallegrave, Fabiana D. Mantese, Rosemari T. Oliveira, Anderson J. M. Andrade, Paulo R. Dalsenter and Augusto Langeloh (2007). Pre- and postnatal toxicity of the commercial glyphosate formulation in Wistar rats. Archives of Toxicology Volume 81, Number 9 (2007), 665-673, DOI: 10.1007/s00204-006-0170-5

Sept 17, 2012

Note To/Note au: Toxicology File

From/De: 2032
Evaluation Officer, TRS-2, HED

Subject/Objet: **Sub. No(s):** 2004-0175
Product Name: Glyphosate products
Active Ingredient: Glyphosate acid and salt forms (GPS)

Action Requested : Screen of Open Literature Studies

Study Title: Morphological damages of a glyphosate-treated keratinocyte cell line revealed by a micro- to nanoscale microscopic investigation.

Summary: Immortalized human HaCaT cell line was incubated in FCS-free medium containing increasing concentrations of glyphosate (0 to 70 mM) for four incubation times (0.5, 4, 18 or 24 hours). IC 50 was 28mM after 4 hour incubation and 53mM after 0.5 hour of treatment. With glyphosate treatment, overproduction of H_20_2 was observed, indicative of oxidative stress. Control cells developed numerous thin adhesion expansions allowing them to spread on substrate and formed uniform layer of similar globular cell shapes. Green-labeled cytoskeleton was more disorganized in glyphosate treated cells as compared to the control cells, it appeared less confined and the cells themselves showed elongated morphology at IC50. At higher cytotoxicity, IC65, serious integrity alteration was noted due to diffuse content of tubulin. Treated cells also presented flattened cell membrane (less protrusions), and cell size was reduced two-fold, while control cells' surface showed multiple regularly densely packed crest-like protrusions. The shrinking of cells and lack of adhesion and loss of cell integrity is characteristic of apoptotic phenomena.

Limitations:

- Induction and visualisation process has not been evaluated for reproducibility and validity, and did not indicate a graded scale of effects. It is difficult to directly correlate *in vitro* effects with *in vivo* toxicity and to determine a novel NOAEL value

- Purity was not specified

FLAG: Glyphosate can induce oxidative stress *in vitro*

Conclusion: This study does not meet the minimum criteria to be eligible for risk assessment of the active ingredient, as outlined in the draft "Guidance for considering and using open literature studies to support human health risk assessment" (US EPA, 2011). The major limitation is non-validated method of cell analysis. This study is categorized as 'invalid' (inappropriate for quantitative or qualitative use in risk assessment because it is of insufficient quality and lacks scientific defensibility).

Reference: Elie-Caille, C., Heu, C., Guyon, C. and Nicod, L. (2010). Morphological damages of a glyphosate-treated keratinocyte cell line revealed by a micro- to nanoscale microscopic investigation. Cell Biol Toxicol. 26: 331-339.

Sept 14, 2012

Note To/Note au: Toxicology File

From/De: 2032
Evaluation Officer, TRS-2, HED

Subject/Objet: **Sub. No(s):** 2004-0175
Product Name: Glyphosate products
Active Ingredient: Glyphosate acid and salt forms (GPS)

Action Requested : Screen of Open Literature Studies

Study Title: Glyphosate-based herbicides are toxic and endocrine disruptors in human cell lines.

Summary: Four different glyphosate-formulations at sub-agricultural doses, with glyphosate (G) as negative control, were tested in human liver HepG2 cells for cytotoxicity (three assays: Alarm Blue®, MTT, Toxilight®), genotoxicity (comet assay), anti-estrogenic effects (on estrogen receptors: ERα, ERβ) and anti-androgenic effects (on androgen receptors, AR) using gene reporter tests. In addition, androgen to estrogen conversion was examined by aromatase activity and mRNA. The Roudup (R) formulations included: Roundup Express ® 7.2g/L of G (R7.2), Bioforce ® or Extra 360 at 360 g/L of G (R360), Grands Travaux ® 400 g/L of G (R400) and Grands Travaux plus ® 450 g/L of G (R450).

Authors concluded that all G-formulations, in contrast to glyphosate alone, induced a rapid decrease in cell viability within 24 hours of exposure, in a dose-dependent fashion to the concentration of formulation, not to G concentration in all cases. The most cytotoxic formulation was R400, which had less glyphosate content than the next most cytotoxic formulation of R450, followed by R360 and R7.2, as determined by measured values of LC50. Initial, statistically significant toxicity was noted at around LC10 (~10%). Data also indicated that DNA of human hepatoma cell line is damaged by G-based herbicide formulation. Around 50% DNA damage was noted in HepG2 cells exposed to 5ppm R400 for 24 hours, showing even more damage (75%) at 10 ppm of R400 (corresponding to 24µM of G), as compared to 35% in negative control and 95% in positive control of Benzo(a)pyrene (50µM, twice the concentration of treatment).

Examining endocrine activity, G alone was always inactive, while all the formulations inhibited androgen to estrogen conversion at concentration below LC50 within 24 hours. This would indicate that the adjuvants in the formulations are responsible for the toxicity, not the active ingredient itself. Non-toxic doses of R450 (60 ppm) significantly activated caspases 3/7 up to 156% in 24 hours, and 765% within 48 hours, indicating induction of apoptosis. Biphasic effects were noted in aromatase mRNA levels, with observed increases of 130-250% and then return to almost normal levels. R400 initiated an inhibition, and then the increase. These effects were neither linear nor proportional to G concentration.

G had no anti-estrogenic activity but was anti-androgenic at sub-agricultural and non-cytotoxic level in MDA-MB453-kb2 cells. Observed disruptions of estrogen and androgen dependent transcriptional activities were quite linear and R-dose dependent (not G-dependent), and within 24 hours of exposure to all formulations. R400 was approximately twice more active on ERβ, and R450 on ERα. All formulations, except R450, appeared to be more anti-androgenic than anti-estrogenic, with inhibitions efficiencies of R400>R450>R360>R7.2 (300-800 times difference in strength).

In conclusion, G-based herbicides present DNA damage and carcinogen, mutagen and repro-toxic and endocrine disrupting effects on human cells, with direct action of G likely amplified by vesicles formed

by adjuvants or detergent-like substances that allow cell penetration, stability and probably change bioavailability and thus metabolism.

Limitations:

- The test substances were glyphosate commercial formulations and glyphosate as negative control.

- The percentages of active ingredient were indicated but purity of glyphosate was not specified. Other components of the formulation were not identified.

Conclusion: This study does not meet the minimum criteria to be eligible for risk assessment of the active ingredient, as outlined in the draft "Guidance for considering and using open literature studies to support human health risk assessment" (US EPA, 2011). The major limitations as discussed above were not characterizing the test substance properly, and experiments focusing on formulations not the active ingredient itself. This study is categorized as 'invalid' (inappropriate for quantitative or qualitative use in risk assessment because it is of insufficient quality and lacks scientific defensibility).

Reference: Gasnier, C., Dumont, C., Benachour, N., Clair, E. and Chagnon, M.-C. (2009). Glyphosate-based herbicides are toxic and endocrine disruptors in human cell lines. Journal of Occupational Medicine and Toxicology, 5:29.

Sept 12, 2012

Note To/Note au: Toxicology File

From/De: 2032
Evaluation Officer, TRS-2, HED

Subject/Objet: **Sub. No(s):** 2004-0175
Product Name: Glyphosate products
Active Ingredient: Glyphosate acid and salt forms (GPS)

Action Requested : **Screen of Open Literature Studies**

Study Title: Dig 1 protects against cell death provoked by glyphosate-based herbicides in human liver cell lines.

Summary: The mechanism of action and possible protection of a new drug, Dig 1, was studied in human hepatic cell lines HepG2 and Hep3B treated with four formulations of glyphosate-based herbicides. Dig 1 (D) contains diluted plant extracts chosen in particular for their digestive detoxification and hepato-protective effects. The four glyphosate formulations were: Express 7.2g/L of glyphosate (G), Bioforce 360 g/L of G (R360), GT 400 g/L of G (R400) and GT+ 450 g/L of G (R450), and all were used at sub-agricultural levels. Mitochondrial succinate dehydrogenase (SD) activity, caspases 3/7 and cytochromes P450 1A1, 1A4 and 2C9 and glutathione-S-transferase (GST) were monitored, as precise biomarkers that could be modified in human hepatocytes. Cells were grown at 37ºC in medium EMEM (M) with 10% serum during 48 hours to 80% confluence in 24-well plates, before being exposed to G-formulations. HepG2 cells have three-fold higher levels of CYP1A1 and GST than Hep3B.

Both liver cell lines showed similar growth rate that was easily disrupted by treatment with glyphosate formulations, however Hep3B cells were 3-5 times more sensitive to R7.2 and R360 over 48 hours. Cell death was estimated by inhibition of SD (mitochondrial metabolism). Mortality of both cell lines increased with concentration and time exposure to all 4 G-formulations. Even though they contain different concentration of G, R360 and R7.2 showed similar toxicities, indicating toxic effects of other ingredients of the formulations. Hep3B cells were much more sensitive to R400 than HepG2 cells, when LC50 was identified. Hepg2 cell viability with the four formulations indicated LC50s at lowest concentration % for R400, R450, R360, R 7.2 and lastly glyphosate, indicating that cytotoxic effect did not vary linearly with dose of glyphosate. It was found that D alone was not toxic (at 2% for up to 72 hours), and that D was able to prevent toxicity when used as pre-treatment. After 24 hour exposure of D and 24 hours exposure of R, D was 43% protective in Hep3B and 55% in HepG2 cell lines,. Protective action was 62% and 89% after 48 hours of D pre-incubation in those cell lines, respectively. However D had no curative effect (no effect when used as post-treatment of R). The efficiency of D protection was observed as early as 6 hours after adding the drug to the cells.

Caspaces 3/7 were activated up to 156% by 24 hours exposure to R and up to 765% by 48h exposure, indicative of early apoptosis. Caspases recover to basal activity in 24 hours with removal of R and incubation in the medium. D did not induce caspaces, but appeared to prevent induction of caspaces by R. Additionally, R did not activate all cytochromes, but was able to enhance CYP3A4 to 240-360% and CYP1A2 to 130-170%. D did not enhance these cytochromes itself, but weakly increased CYP2C9 up to 140%, when added after R treatment, as compared to M control. When D was added before R treatment, no cytochrome activity was stimulated, and CYP2C9 was weakly inhibited (40%). However, D did not modify the effect of R inhibiting GST (~50%).

It was concluded that D penetrates the cells and not just forms a shield that prevents R from penetrating since D had intracellular action, as noted by its role in preventing caspases 3/7 activation and CYP3A4 enhancement by R.

Limitations:

- The test substances were glyphosate commercial formulations and a new drug Dig 1.
- The percentages of active ingredient were indicated but purity of glyphosate was not specified. Other components of the formulation were not identified.
- The study focused more on the protective effect of Dig 1, than toxicity of glyphosate

Conclusion: This study does not meet the minimum criteria to be eligible for risk assessment of the active ingredient, as outlined in the draft "Guidance for considering and using open literature studies to support human health risk assessment" (US EPA, 2011). The major limitations as discussed above were not characterizing the test substance properly. This study is categorized as 'invalid' (inappropriate for quantitative or qualitative use in risk assessment because it is of insufficient quality and lacks scientific defensibility).

Reference: Gasnier, C., Benachour, N., Clair, E., Travert, C., Langlois, F., Laurent, C., Decroix-Laporte, C., and Seralini, G-E. (2005). Dig 1 protects against cell death provoked by glyphosate-based herbicides in human liver cell lines. Journal of Occupational Medicine and Toxicology, 5:29.

August 27, 2012

Note To/Note au: Toxicology File

From/De: 2032
Evaluation Officer, TRS-2, HED

Subject/Objet: **Sub. No(s):** 2004-0175
Product Name: Glyphosate products
Active Ingredient: Glyphosate acid and salt forms (GPS)

Action Requested : **Screen of Open Literature Studies**

Study Title: Mechanism of Toxicity of Commercial Glyphosate Formulations: How Important is the Surfactant?

Summary: Typically, ethoxylated tallowamine surfactant is used at 10-20% in formulation with 41% glyphosate and various minor ingredients (dye, silicone antifoam). It has been suggested that surfactant or glyphosate may impede mitochondrial function. Published results (2001) indicated that glyphosate was an "endocrine disruptor" showing inhibition of steroidogenesis in cultured Sertoli cells. In contrast, this finding was attributed by the authors to direct cytotoxicity of the surfactant. Decreased stereoidogenesis was coincident with mitochondrial membrane damage and subsequent loss of mitochondrial membrane potential. The surfactant mediated uncoupling of mitochondrial oxidative phosphorylation was linked with much of a clinical toxicity observed following ingestion of herbicide formulation containing low-toxicity active ingredient, such as glyphosate.

Limitations:

- Only an abstract is available
- The test substance is poorly characterized

Conclusion: The abstract does not meet the minimum criteria to be eligible for risk assessment of the active ingredient, as outlined in the draft "Guidance for considering and using open literature studies to support human health risk assessment" (US EPA, 2011). The major limitation was lack of reported data and poor characterization of test substance. This study is categorized as 'invalid' (inappropriate for quantitative or qualitative use in risk assessment because it is of insufficient quality and lacks scientific defensibility).

Reference: Abstracts of the European Association of Poisons Centres and Clinical Toxicologists XXV International Congress (2005). Clinical Toxicology, 43:387–538
DOI: 10.1080/07313820500207624
Goldstein DA, Farmer DL, Levine SL, Garnett RP. 45. Mechanism of Toxicity of Commercial Glyphosate Formulatons: How Important is the Surfactant?
The Monsanto Company, St. Louis, Missouri, USA and Monsanto Europe SA, Brussels, Belgium.
http://www.eapcct.org/publicfile.php?folder=congress&file=Abstracts_Berlin.pdf

Note To/Note au: Toxicology File

From/De: John Taylor
Senior Re- evaluation Officer, TRS-2, HED

Subject/Objet: **Sub. No(s):** 2004-0175
Product Name: Glyphosate products
Active Ingredient: Glyphosate acid and salt forms (GPS)

Action Requested : Screen/Review of Open Literature Studies

Study Title: (6) Prepubertal exposure to commercial formulation of the herbicide glyphosate alters testosterone levels and testicular morphology

Summary: The glyphosate formulation, Roundup Transorb, was administered orally as an aqueous suspension to newly weaned male Wistar rats. Four groups of 16-18 animals were treated at doses of 0, 5, 50 or 250 mg/kg bw/day from PND23 to PND53. Age and weight at preputial separation was recorded. At 53 days the animals were sacrificed: serum was collected via cardiac puncture for hormone analysis; testes and adrenal glands were weighed and prepared for histological examination.

No significant differences in body weight were noted among groups, however a significant delay in pubertal age, indicated by the timing of preputial separation, occurred at the two highest doses. Adrenal and testicular weights in the highest dose group were significantly increased compared to the control group. Differences between treated and control animals were noted in testicular seminiferous tubule morphology: treated animals had an increase in the luminal diameter and a corresponding decrease in tubular epithelium height, indicating a reduction in germ cells. The concentration of serum testosterone was significantly reduced in treated groups.

These results suggest that the commercial formulation of glyphosate can affect the metabolism of hormones causing disturbances in the reproductive development of rats when exposure occurs in the period leading up to puberty.

Limitations:

- The test substance was a glyphosate-based commercial formulation composed of 480 g/l of glyphosate, 648 g/l of isopropylamine salt (of glyphosate) and 594 g/l of inert ingredients. No description of the inert ingredients was given.

- Histology was restricted to an examination of the testes and adrenal glands.

Conclusions: This study does not meet the minimum criteria to be eligible for risk assessment of the active ingredient, as outlined in the draft "Guidance for considering and using open literature studies to support human health risk assessment" (US EPA, 2011). The major limitation as discussed above was not using the active ingredient as the test substance. This study is categorized as 'invalid' (inappropriate for quantitative or qualitative use in risk assessment).

Reference: Romano, R.M., Romano, M.A., Bernardi, M.M., Furtado, P.V. & Oliveira, C.A. (2010) Prepubertal exposure to commercial formulation of the herbicide glyphosate alters testosterone levels and testicular morphology. Arch. Toxicol. 84(4):309-317.

Study Title: (7) Effect of the herbicide glyphosate on liver lipoperoxidation in pregnant rats and their foetuses

Summary: Glyphosate solutions were prepared from the commercial product, Herbicygon (Argentina). Pregnant Wistar rats were divided into two groups, 8 rats/group. Group I was given tap water, and group II was given tap water containing 1% glyphosate from the commercial product, from GD1 to GD23. At GD 23 days the animals were sacrificed: each fetus was examined and weighed; serum was collected via cardiac puncture; maternal and fetal livers were collected. The levels of lipid peroxidation products, mainly malonaldehyde (MDA), were determined in maternal blood serum, and in homogenates of maternal and fetal liver. Activities of the antioxidant enzymes, glutathione peroxidase (GPx), catalase, and superoxide dismutase (SOD) were also determined in homogenates of maternal and fetal liver.

Food and water consumption, maternal body weight gain and liver weight were lower in glyphosate-treated dams compared to the controls. Lipoperoxidation was higher in both the maternal and fetal livers in the glyphosate-treated group and this increase was higher in the liver of foetuses than in the dams. Glyphosate exposure led to increased activity of GPx in fetal livers, in contrast to no change in SOD or catalase activity.

On the basis of the lipoperoxidation biomarkers tested, glyphosate-containing formulation ingestion during pregnancy has the potential to create oxidative stress in the fetus and dam.

Limitations:

- The test substance was a glyphosate-based commercial formulation, Herbicygon marketed in Argentina by M.F.L., S.R.L. The % active ingredient and purity were not stated.

- Histopathology was not performed on the maternal and fetal livers that were collected. No other organs were examined.

- A single treated group, rather than a range of dosage groups, was compared to the control. No explanation is given for the dosage chosen.

Conclusions: This study does not meet the minimum criteria to be eligible for risk assessment of the active ingredient, as outlined in the draft "Guidance for considering and using open literature studies to support human health risk assessment" (US EPA, 2011). The major limitations, as discussed above include the lack of information on the purity and % active ingredient of the test substance and the use of a single dose level for the test substance. This study is categorized as 'invalid' (inappropriate for quantitative or qualitative use in risk assessment).

Reference: Beuret, C.J., Zirulnik, F, & Giménez, M.S. (2005) Effect of the herbicide glyphosate on liver lipoperoxidation in pregnant rats and their foetuses. Reproductive Toxicology 19:501-504.

Study Title: (8) A generational study of glyphosate-tolerant soybeans on mouse fetal, postnatal, pubertal and adult testicular development

Summary: C57BL/6J female mice, mated with C3H/HeJ male mice, were feed a glyphosate-tolerant soybean-based diet—from a crop which had been treated with commercial levels of glyphosate—or a conventional soybean-based diet, through gestation and lactation. After weaning, the young male mice were maintained on the respective diets. At 8, 16, 26, 32, 63 and 87 days after birth, three male mice and an adult reference mouse were killed, the testes surgically removed, and the cell population measured by flow cytometry. A multi-generational study was also conducted in which mice were fed the transgenic soybean diet or the conventional diet over four generations; testicular cell population measurements were taken on the 4th generation mice at the time periods used in the short-term study.

There were no differences in the percentages of testicular cell populations (haploid, diploid, and tetraploid) between the transgenic soybean-fed mice and those on the conventional diet. Additionally, there were no differences in litter sizes and body weights of the two groups.

It was concluded that the glyphosate-tolerant soybeans did not have a negative effect on fetal, postnatal, pubertal or adult testicular development or body growth.

Limitations:

- This study is concerned with the effect of a glyphosate-tolerant soybean-based diet compared to a conventional soybean-based diet during development and maturation in mice. It does not deal with the effect of glyphosate itself as an active ingredient. While the transgenic soybean crop was treated with glyphosate there is no indication that residues of glyphosate remain, nor are there any details of the actual crop treatment.

Conclusions: This study does not meet the minimum criteria to be eligible for risk assessment of the active ingredient, as outlined in the draft "Guidance for considering and using open literature studies to support human health risk assessment" (US EPA, 2011). The purpose of the study is to determine if adverse effects result from exposure to transgenic (glyphosate-tolerant) plant material; it is not to determine if adverse effects result from exposure to the active ingredient, glyphosate. This study is categorized as 'invalid' (inappropriate for quantitative or qualitative use in risk assessment).

Reference: Brake, D.G. and Evenson, D.P. (2004) A generational study of glyphosate-tolerant soybeans on mouse fetal, postnatal, pubertal and adult testicular development. Food Chem. Toxicol. 42(1):29-36.

Study Title: (9) Reproductive toxicity studies with octamethyl cyclotetrasiloxane in female rats using various exposure regimens

Summary: Previous studies have shown that pregnant Sprague-Dawley rats exposed to the vapour of octamethyl cyclotetrasiloxane (D4) had decreases in litter size and number of uterine implants. The present study attempted to identify the time of greatest sensitivity for these adverse reproductive effects. Female Sprague-Dawley rats were exposed to 700 ppm D4 by whole body inhalation as follows: (1) 28 continuous days prior to mating, during mating and gestation to GD19; (2) 28 continuous days followed by 3 days without exposure prior to mating; (3) 3 days prior to mating through GD3; and (4) from GD 2 through GD 5. Laparohysterectomies were performed on GD20 and corpora lutea, early and late resorptions, and viable foetuses were counted.

Reduced fetal numbers were noted in situations (1) and (3) with modest numerical decreases seen in corpora lutea, and an increase in pre- and post-implantation losses. No effects were noted in (2) and (4).

The results suggest that reproductive processes around the time of ovulation are being affected. The lack of effect when treatment is stopped 3 days prior to mating indicates the treatment does not cause permanent reproductive impairment.

Limitations:

- This study does not deal with the active substance glyphosate.
- The report is an abstract of a conference poster.

Conclusions: This study does not meet the minimum criteria to be eligible for risk assessment of the active ingredient, as outlined in the draft "Guidance for considering and using open literature studies to support human health risk assessment" (US EPA, 2011). The study does not deal with the active ingredient and no experimental details are provided. This study is categorized as 'invalid' (inappropriate for quantitative or qualitative use in risk assessment).

Reference: Reynolds, V.L., DeSesso, J.M., Mast, R.W., Stump, D.G. & Holson, J.F. (1998) Reproductive toxicity studies with octamethyl cyclotetrasiloxane in female rats using various exposure regimens. Toxicology Letters 95, Supplement 1: 216

Study Title: (11) Effect of the herbicide glyphosate on enzymatic activity in pregnant rats and their foetuses

Summary: Glyphosate solutions were prepared from the commercial product, Herbicygon (Argentina). Pregnant Wistar rats were divided into three groups, 8 rats/group. Group I was given tap water, group II was given tap water containing 0.5% glyphosate, and group III was given tap water containing 1% glyphosate, from GD1 to GD21. A fourth group, designated low food and low water, was given reduced quantities of food and water in the second week to mimic the reduction in food and water consumption noted in group III, in order to establish if effects in group III were due to glyphosate or were due to the food/water reduction. At GD 21 the animals were sacrificed: each fetus was examined and weighed; maternal and fetal livers, hearts and brains were collected for analysis. Cytosolic fractions were isolated from homogenates of these organs and the activities of isocitrate dehydrogenase (ICD), gluscose-6-phosphate dehydrogenase (G6PD), and malic dehydrogenase (MD) were determined.

Food and water consumption decreased with both doses of glyphosate. Maternal body weight gain and liver weight were lower in 1% glyphosate-treated dams compared to the controls. A number of alterations in enzyme activity were noted with glyphosate treatment.

In the pregnant females, ICD increased with the 1% glyphosate treatment in the liver, heart and brain, but with the 0.5% glyphosate treatment there was a decrease in the liver, an increase in the heart and no change in the brain. In the foetuses, ICD with the 1 % glyphosate treatment decreased in the liver, remained the same in the heart and increased in the brain, while with 0.5% glyphosate there was no difference in the liver and heart, and an increase in the brain.

In the pregnant females, with the 1% glyphosate treatment, G6PD remained the same in the liver and brain, and increased in the heart, while with the 0.5% glyphosate treatment there was a decrease in the liver, and no change in the heart and brain. In the foetuses, with the 1% glyphosate treatment, G6PD remained the same in the liver and heart and increased in the brain, while with the 0.5% glyphosate treatment there was no difference in the liver and brain, and an increase in the heart.

In the pregnant females, with glyphosate treatment at either dose, MD activity in the liver, brain, and heart remained the same. In the foetuses, with the 1% glyphosate treatment, MD remained the same in the liver, decreased in the heart, and increased in the brain, while with the 0.5% glyphosate treatment there was no difference in the liver and brain, and a decrease in the heart.

The enzymatic activity in the low food and low water group did not show any significant differences from the control for any of the enzymes or organs.

The study demonstrated that the enzymatic activity of the dehydrogenases of both pregnant rats and their foetuses could be altered by glyphosate formulation treatment. The effect of glyphosate formulation depended on its concentration and was specific for each enzyme in each of the different organs.

Limitations:

- The test substance was a glyphosate-based commercial product, Herbicygon marketed in Argentina by M.F.L., S.R.L. The % active ingredient and purity were not stated.

- Two dosage groups were used rather than a range. A few additional dosages would help to clarify the considerable variation in response at different concentrations.

Conclusions: This study does not meet the minimum criteria to be eligible for risk assessment of the active ingredient, as outlined in the draft "Guidance for considering and using open literature studies to support human health risk assessment" (US EPA, 2011). The major limitation is the lack of information on the purity and % active ingredient of the test substance. This study is categorized as 'invalid' (inappropriate for quantitative or qualitative use in risk assessment).

Reference: Daruich, J., Zirulnick, F., & Giménez, M.S. (2001) Effect of the herbicide glyphosate on enzymatic activity in pregnant rats and their foetuses. Environmental Research Section A 85:226-231.

Study Title: (12) The teratogenic potential of the herbicide glyphosate-Roundup in Wistar rats

Summary: Roundup solutions were prepared from the commercial product, Roundup (Brazil; 36% glyphosate and 18% polyoxyethyleneamine). Pregnant Wistar rats were divided into four groups, 15 rats/group. The control group received distilled water and the experimental groups received 500, 750 or 1000 mg/kg Roundup. Treatments were administered by gavage in a volume of 10 ml/kg from GD 6 to GD 15. At GD 21 days the animals were submitted to caesarean section and sacrificed. The number of corpora lutea, implantation sites, living and dead foetuses and resorptions were recorded. The weight and gender of the foetuses were determined, and foetuses were examined for external malformations and skeletal alterations. The organs of the dams were removed and weighed.

At the high dose there was a 50% mortality rate among the dams. There were no significant differences in weight gain, food intake, water intake, or relative weight of the maternal organs between the groups. The number of foetuses, corpora lutea, implantation sites and embryo resorptions were similar for all groups.

In the foetuses, there were no significant differences in weight, male:female sex ratio or external malformations. Foetuses in the groups exposed to Roundup had a significantly greater incidence of skeletal alterations compared to the control. The percentage of altered foetuses was 15.4, 33.1, 42.0 and 57.3 for 0, 500, 750, and 1000 mg/kg Roundup respectively. The most frequent skeletal alterations observed were incomplete skull ossification and enlarged fontanel which occurred more frequently in the experimental groups and in a dose-related fashion. While multiple alterations were significantly higher in the treated groups, the pattern for specific alterations was not consistently dose-related. The general occurrence of incomplete ossification and bipartite sternebra was highest in the 500 mg/kg Roundup group. Bipartite interparietal and unossified hind phalanges were significantly more frequent in the 500 and 750 mg/kg Roundup groups. Incomplete ossification of squama and absence of caudal vertebrae appeared more frequently in the 750 and 1000 mg/kg groups. A number of alterations, such as incomplete ossification of tibia, fibula and femur, and unossified metatarsal bones, were significant only at 750 mg/kg.

The study shows that Roundup is toxic to pregnant rats and induces developmental retardation of the fetal skeleton.

Limitations:

- The test substance was the glyphosate-based commercial product, Roundup, as marketed in Brazil, rather than the active ingredient alone. Inert ingredients, in addition to the listed 36% glyphosate and 18% polyoxyethyleneamine, were not stated.

Conclusions: This study does not meet the minimum criteria to be eligible for risk assessment of the active ingredient, as outlined in the draft "Guidance for considering and using open literature studies to support human health risk assessment" (US EPA, 2011). The major limitation is the use of a formulated product rather than reagent grade glyphosate of a known purity. This study is categorized as 'invalid' (inappropriate for quantitative or qualitative use in risk assessment).

Reference: Dallegrave, E., Mantese, F.D., Coelho, R.S., Pereira, J.D., Dalsenter, P.R. & Langeloh, A. (2003) The teratogenic potential of the herbicide glyphosate-Roundup in Wistar rats. Toxicol. Lett. 142(1-2):45-52

Study Title: (14) The impact of simultaneous intoxication with agrochemicals on the antioxidant defense system in rat

Summary: Nine groups of 4 male Wistar rats were given intraperitoneal (i.p.) injections of dimethoate, glyphosate and zineb, either alone or in combination, three times a week for a total of 5 weeks. Doses used for each pesticide were in the range 1/50 to 1/250 LD_{50}. At the end of the study the animals were sacrificed and blood, livers and testes were collected. Various biomarkers for oxidative status and cell damage were measured. Nitrates and nitrites ([NO_x]) were measured as the main end-metabolic products of nitric oxide and peroxinitrite anion. Total glutathione and the activities of antioxidant defense enzymes such as catalase (CAT), superoxide dismutase (SOD) and the glutathione-dependent enzymes [glutathione peroxidase (GP_x), glutathione reductase (GR) and glutathione-S-transferase (GST)] were measured to determine the antioxidant defense status. Protein carbonyls were analysed as a biomarker of oxidative damage to proteins. The thiobarbituric acid-reactive substances (TBARS) were determined as biomarkers of oxidative damage to lipids. Hormone levels in plasma samples and testicular homogenates were measured by radioimmunoassay to assess androgenic function.

Pesticide exposure did not produce clinical signs of toxicity, nor did it affect animal behaviour or body weight. Plasma [NO_x] concentrations were significantly higher (30 to 80-fold) in treated groups than in the control group; results in liver and testes were similar, but less pronounced. The concentration of glutathione in the plasma increased by 54% to 72% in all treated groups except in the zineb alone; a similar pattern was observed in the testes except the concentration of glutathione increased in all treated groups except in the glyphosate alone. SOD activity was lower in treated groups compared to the control group; however, only in the liver were those differences statistically significant. CAT activity was not modified in liver homogenates, but was significantly decreased, by 30 to 70%, in the testes of treated animals. The activities of GP_x or GST were unchanged, while GR was less active, in the liver homogenates of treated animals; GP_x, GR and GST activity increased in in testes of treated animals.

Oxidative damage to proteins, measured by the presence of carbonyl groups in the side chains of oxidized amino acid residues, occurred in almost all treated groups. Significantly higher levels of protein carbonyls (PCOs) were found in the plasma of treated groups, except for the glyphosate alone, or the zineb plus glyphosate, and in testes homogenates of all treated groups. A significant increase in lipid peroxidation levels in treated groups, indicated by an increase in TBARS, was noted in liver, testes and plasma. Measurements of hormones in plasma and testicular homogenates demonstrated that rats treated with dimethoate, either alone or in combination with the other pesticides, had altered hormone levels. Plasma from dimethoate-treated rats contained less free and bound testosterone by approximately 20%. A decrease of approximately 50% in testosterone production was noted in testicular homogenates. Plasma estradiol concentration decreased in dimethoate-treated rats by approximately 30%, while LH and FSH increased by 58% and 76% respectively. Neither glyphosate alone nor zineb alone had an effect on hormone levels.

Treatment with the pesticides increased oxidative stress and damage biomarker levels, altered the antioxidant defense system and altered hormone levels. The results demonstrate that testicular tissue is susceptible to oxidative stress induced by low doses of pesticides and the effects are more pronounced when pesticides are administered in combination.

Limitations:

- The % active ingredient and purity were not stated for the individual pesticides.
- The method of dosing was via i.p. injection, which is not a mode of dosing relevant to human health.

- A single dose level for each particular pesticide was compared to the control. All other treatments involved combinations of more than one pesticide.

Conclusions: This study does not meet the minimum criteria to be eligible for risk assessment of the active ingredient, as outlined in the draft "Guidance for considering and using open literature studies to support human health risk assessment" (US EPA, 2011). The major limitations, as discussed above include the lack of information on the purity and % active ingredient of the test substance, use of i.p. injection and the use of a single dose level for the test substance. This study is categorized as 'invalid' (inappropriate for quantitative or qualitative use in risk assessment).

Reference: Astiz, M., de Alaniz, M.J.T., & Marra, C.A. (2009) The impact of simultaneous intoxication with agrochemicals on the antioxidant defense system in rat. Pestic. Biochem. Physiol. 94:93-99.

Study Title: (16) Oxidative stress responses of rats exposed to Roundup and its active ingredient glyphosate

Summary: Three groups of 16 adult male albino rats were given intraperitoneal (i.p.) injections of 1 ml saline/kg, 269.9 mg/kg of Roundup, or 134.95 mg/kg of glyphosate in a volume of 1 ml/kg every second day for 14 days. Doses used for each pesticide were within the limits of the NOAEL and equivalent to 1/4 LD_{50}. Blood samples were collected after 1 and 2 weeks; individual animal body weight and organ weights were recorded weekly. Hepatic toxicity was monitored by the analysis of serum AST, ALT and ALP activities as well as total protein, albumin, triglycerides and cholesterol. Creatinine and urea levels were measured as biochemical markers of kidney damage. The effect of glyphosate/Roundup on hepatic reduced glutathione (GSH) and lipid peroxidation levels was examined as an index of antioxidant status and oxidative stress. Serum nitric oxide (NO) and alpha tumour necrosis factor (TNF-α) were also measured.

No significant changes in body weight were noted at 2 weeks; the liver weight increased at 1 week with Round–up treatment, but this was no longer in evidence at 2 weeks. At 2 weeks AST, ALT and ALP activities in liver homogenates were significantly greater in treated animals than in controls. ALP levels were approximately 2-fold greater than controls at 2 weeks in Roundup–treated animals, while in glyphosate-treated animals the increase was less (app. 1.2-fold). Roundup/glyphosate treatments did not significantly affect total protein and albumin. At 2 weeks, serum creatinine, urea and uric acid levels in treated animals were significantly higher than in control animals. Roundup/glyphosate treatments significantly increased cholesterol and triglyceride levels compared to the control.

The level of GSH in the liver of treated rats was significantly decreased after 1 week of treatment compared to the control, with a greater effect noted at 2 weeks. The level of LPO was increased in the liver of treated rats after 2 weeks; the increase in the glyphosate-treated group was significantly greater than the increase in the Roundup-treated group. Serum NO levels were significantly increased after 2 weeks of treatment. Serum TNF-α levels increased at both 1 week and 2 weeks in the treated animals.

These results characterize Roundup as a probable antioxidant disruptor with a greater effect than the active ingredient itself (glyphosate). Exposure to sub-lethal concentrations of Roundup promoted an increase in hepatic LPO and TNF-α, indicating a typical response to stress and inflammation. The depletion of hepatic GSH indicates the activation of antioxidant defenses, probably due to increased hydrogen peroxide generation. Roundup also induces a variety of liver and kidney biochemical alterations that might impair normal organ functioning.

Limitations:

- The % active ingredient and purity were not stated for the individual pesticides.
- The method of dosing was via i.p. injection, which is not a mode of dosing relevant to human health.
- A single dose level for each particular pesticide was compared to the control. All other treatments involved combinations of more than one pesticide.

Conclusions: This study does not meet the minimum criteria to be eligible for risk assessment of the active ingredient, as outlined in the draft "Guidance for considering and using open literature studies to support human health risk assessment" (US EPA, 2011). The major limitations, as discussed above include the lack of information on the purity and % active ingredient of the test substance, use of i.p.

injection and the use of a single dose level for the test substance. This study is categorized as 'invalid' (inappropriate for quantitative or qualitative use in risk assessment).

Reference: El-Shenawy, N.S. (2009) Oxidative stress responses of rats exposed to Roundup and its active ingredient glyphosate. Environ. Toxicol. Pharmacol. 28(3):379-385.

Study Title: (18) Studies on glyphosate-induced carcinogenicity in mouse skin: a proteomic approach

Summary: A commercial formulation of glyphosate, Roundup Original (glyphosate 41%, POEA ≈ 15%), was used rather than the active ingredient. The study was divided into two parts: a carcinogenicity study, and a proteomic study.

In the carcinogenicity study, a 2-stage mouse skin tumour initiation-promotion protocol was used. The tumor initiator, 7,12-dimethylbenz[a]anthracene (DMBA), the tumour promoter, 12-o-tetradeccanoylphorbol-13-acetate (TPA), and Roundup, were applied to the skin of male, Swiss albino mice separately or in combination for a total of 8 groups of 20 animals each. Animals from each group were examined each week for gross morphological changes including body weight changes, and development and volume of squamous cell papillomas (tumours) on the treated skin. Animals were sacrificed after 32 weeks. Roundup demonstrated potential as a tumour promoter in the presence of the tumour initiator, DMBA, but did not produce neoplastic development in the absence of a tumour initiator.

In the proteomic study, four groups of mice were used: an untreated control group, and groups with single topical applications of DMBA, TPA, or Roundup. Twenty-four hours after treatment, the animals were sacrificed and skin tissue from the treated area was excised. Proteins in the excised skin were analysed by 2-dimesional gel electrophoresis. Twenty-two spots were differentially expressed, exhibiting a greater than a 2-fold change between values of treated and control animals, and were examined further by mass spectrometry. Nine proteins, known to be involved in apoptosis and growth inhibition, anti-oxidation, energy metabolism, angiogenesis, calcium binding and protein biosynthesis processes, were common to both Roundup and TPA-treated mouse-skin. These proteins were translation elongation factor eEF-1 alpha chain (eEF1A1), carbonic anhydrase 3 (CA III), annexin II, calcyclin, fab fragment of anti-VEGF antibody, peroxiredoxin-2 (PRX II), superoxide dismutase [Cu-Zn] (SOD 1), stefin A3, and calgranulin-B. Up-regulation of calcyclin and calgranulin-B, and down-regulation of SOD 1 in the Roundup and TPA-treated groups compared to controls were shown by immunoblotting.

These results suggest that Roundup has tumour promoting potential in skin carcinogenesis and its mechanism seems to be similar to TPA. The proteins, calcyclin, calgranulin-B and SOD 1, were closely associated with the tumour-promoting activity of Roundup treatment and may be useful as early biomarkers for skin carcinogenesis.

Limitations:

- A commercial formulation of glyphosate, Roundup Original (glyphosate as the isopropylamine salt 41%, POEA ≈ 15%), was used rather than the active ingredient. Inert ingredients were not indicated.

- The study does not follow a standard test guideline.

Conclusions: This study does not meet the minimum criteria to be eligible for risk assessment of the active ingredient, as outlined in the draft "Guidance for considering and using open literature studies to support human health risk assessment" (US EPA, 2011). The major limitation, as discussed above, is the use of a formulated product instead of the active ingredient. This study is categorized as 'invalid' (inappropriate for quantitative or qualitative use in risk assessment).

Reference: George, J., Prasad, S., Mahmood, Z. & Shukla, Y. (2010) Studies on glyphosate-induced carcinogenicity in mouse skin: a proteomic approach. J. Proteomics 73(5):951-964.

Study Title: (19) Genotoxic potential of glyphosate formulations: mode-of-action investigations

Summary: This study repeated and evaluated a number of previous studies which had shown that glyphosate-containing herbicide formulations (GCHF) administered intraperitoneally could induce genotoxic effects. Male Crl:CD-1(ICR)BR mice, 8-10 per group, given a single dose of GCHF in a volume of 10 ml/kg bw, were compared to an appropriate control group, in 4 assays: (1) GCHF (600 mg/kg) in isotonic saline by i.p. injection; animals sacrificed at 4 or 24 hours. (2) GCHF (600 mg/kg) in a dimethylsulfoxide/olive oil mixture by i.p. injection; animals sacrificed at 24 hours. (3) GCHF (600 mg/kg) without glyphosate in a dimethylsulfoxide/olive oil mixture by i.p. injection; animals sacrificed at 24 hours. (4) GCHF (900 mg/kg) in isotonic saline by i.p. injection; animals sacrificed at 24 hours, and GCHF (600 mg/kg) in a dimethylsulfoxide/olive oil mixture by oral gavage; animals sacrificed at 24 hours. Following sacrifice, blood was collected for clinical chemistry; kidneys and livers were removed and portions were fixed for sectioning or frozen and used for subsequent assays of 8-hydroxydeoxyguanosine (8-OHdG) and NADPH menadione oxidoreductase (NMO) mRNA.

Intraperitoneal injection of GCHF at 600 mg/kg produced a substantial increase in ALT, AST and LDH at 4 hours which had returned to near control values by 24 hours; in mice given 900 mg/kg GCHF values were still high at 24 hours. Several microscopic changes occurred in kidneys and livers given the GCHF at the 900 mg/kg dose level. Changes in the kidneys included vacuolization of cortical tubules, degeneration and necrosis in the medulla, and acute inflammation of the renal capsule. Hepatic changes included a generalized increase in hepatocellular vacuolization, subcapsular necrosis and subcapsular hepatocellular vacuolization. A statistically significant increase in NMO, evidence of oxidative stress, was seen in kidneys of animals given 900 mg/kg, but not 600 mg/kg GCHF.

Intraperitoneal injection of DMSO/olive oil mixture alone did not produce any significant evidence of toxicity; however, the GCHF/DMSO/olive oil mixture at 600 mg/kg produced a significant effect. Absolute and relative kidney and liver weights were reduced. Serum ALT, AST, LDH, BUN and SDH levels in treated animals increased 151-1065%. Pathological examination revealed several changes in the capsule or subcapsular tissue in both livers and kidneys. Oxidative stress was observed in the kidneys as indicated by a statistically significant increase in NMO.

A comparison of the effects, following i.p. injection, of the GCHF/DMSO/olive oil mixture and the formulation blank, containing all of the same components as the GCHF/DMSO/olive oil mixture except glyphosate, showed that the observed reductions in organ weights, and altered clinical chemistry values were similar for both. Administration of the GCHF/DMSO/olive oil mixture by the oral route of exposure did not produce toxicity.

The results show that high-dose i.p. administration of a GCHF produced significant liver and kidney toxicity. The effect appears to be due to the formulation components rather than the glyphosate since a formulation blank was equally toxic. The methodology involved in injecting the GCHF may be inducing secondary effects mediated by local toxicity rather than genotoxicity. There was no evidence of adverse effects following oral administration of GCHF. It is concluded that glyphosate and GCHF are not genotoxic under exposure conditions that are relevant to human health.

Limitations:

- A commercial formulation of glyphosate, Roundup (glyphosate as the isopropylamine salt ≈ 30%, an alkyl sulphate surfactant), was used rather than the active ingredient. Other inert ingredients were not named.

- The study does not follow a standard test guideline and was largely concerned with intraperitoneal injection of test material which is not relevant to human health concerns.

Conclusions: This study does not meet the minimum criteria to be eligible for risk assessment of the active ingredient, as outlined in the draft "Guidance for considering and using open literature studies to support human health risk assessment" (US EPA, 2011). The major limitation, as discussed above, is the use of a formulated product instead of the active ingredient. This study is categorized as 'invalid' (inappropriate for quantitative or qualitative use in risk).

Reference: Heydens, W.F., Healy, C.E., Hotz, K.J., Kier, L.D., Martens, M.A., Wilson, A.G.E., & Farmer, D.R. (2008) Genotoxic potential of glyphosate formulations: mode-of-action investigations. J. Agric. Food Chem. 56(4):1517-1523.

Study Title: (21) The effect of sub-acute and sub-chronic exposure of rats to the glyphosate-based herbicide Roundup

Summary: The glyphosate formulation, Roundup, was administered orally to 90-day old Wistar rats. Groups of male or female animals (size of groups not stated) were treated at doses of 0, 56 or 560 mg/kg bw/day for either 5 weeks or 13 weeks. Water and food consumption was recorded daily and body weight weekly. At the end of the treatment period the animals were sacrificed and blood samples and livers were taken for biochemical and histopathological analysis.

Clinical symptoms in treated animals included reduced appetite and activity, increasing weakness, diarrhea and collapse (details not given). There were no statistical differences in liver weights, body weight gains, or food and water consumption between control and treated groups. Slight differences in levels of ALT, AST, LDH, lipoprotein (LDL, HDL), cholesterol and creatinine were noted between the sera of treated and control groups, however these differences were not statistically significant. Histopathological effects were found in all treated groups, and included mononuclear cell infiltration, apoptosis of some hepatocytes, focal necrosis, and congestion and swelling of hepatocytes.

Based on the histopathological effects in treated rats, oral doses of the glyphosate formulation Roundup are toxic to the rat liver.

Limitations:

- A commercial formulation of glyphosate, Roundup (360 g/L glyphosate, 18% polyoxyethylene alkylamine), was used rather than the active ingredient. Inert ingredients were not indicated.
- The study does not follow a standard test guideline.
- This paper is poorly written. A number of experimental details are left out, i.e., number of animals/group, method of administering dose, etc. Discussion of results is vague with reference to a variety of other experiments rather than the results of the present study. Very few statistically significant effects occur: in the analysis of the sera for enzymes and other factors it is indicted that "mild effects" or mild differences" occur, however none of these effects or differences, appear to be statistically significant.

Conclusions: This study does not meet the minimum criteria to be eligible for risk assessment of the active ingredient, as outlined in the draft "Guidance for considering and using open literature studies to support human health risk assessment" (US EPA, 2011). The major limitation, as discussed above, is the use of a formulated product instead of the active ingredient. This study is categorized as 'invalid' (inappropriate for quantitative or qualitative use in risk).

Reference: Çağlar, S. & Kolankaya, D. (2008) The effect of sub-acute and sub-chronic exposure of rats to the glyphosate-based herbicide Roundup. Environ. Toxicol. Pharmacol. 25(1):57-62.

Study Title: (22) The effects of sub-chronic exposure of Wistar rats to the herbicide Glyphosate-Biocarb®

Summary: The glyphosate formulation, Glyphosate-Biocarb, was administered orally to 90-day old male Wistar rats. Groups of 16 (control) or 14 (treated) animals were dosed with 0, 4.87, 48.7, or 487 mg/kg bw by oral gavage in a volume of 0.5 ml/kg every 2 days for 75 days. At the end of the treatment period the animals were sacrificed and blood samples and livers were taken for biochemical and histopathological analysis.

Hepatic toxicity was monitored by quantitative analysis of ALT and AST activities. Statistically significant increases were noted in ALT at all doses (to about 2-fold) and in AST at the two highest doses (to about 1.5-fold). Histological observation of the liver showed an increase in the number of Kupffer cells in hepatic sinusoids and an increase in the deposition of reticulin fibers at the highest dose only.

The results indicate that Glyphosate-Biocarb may induce hepatic histological changes as well as AST and ALT leakage from liver to serum.

Limitations:

- A commercial formulation of glyphosate, Roundup (360 g/L glyphosate, 18% polyoxyethylene alkylamine), was used rather than the active ingredient. Inert ingredients were not indicated.

- The study does not follow a standard test guideline.

Conclusions: This study does not meet the minimum criteria to be eligible for risk assessment of the active ingredient, as outlined in the draft "Guidance for considering and using open literature studies to support human health risk assessment" (US EPA, 2011). The major limitation, as discussed above, is the use of a formulated product instead of the active ingredient. This study is categorized as 'invalid' (inappropriate for quantitative or qualitative use in risk assessment).

Reference: Benedetti, A.L., Vituri, C.de L., Trentin, A.G., Domingues, M.A.C.D., and Alvarez-Silva, M.. (2004) The effects of sub-chronic exposure of Wistar rats to the herbicide Glyphosate-Biocarb®. Toxicol. Lett. 153(2):227-232.

Study Title: (32) Clastogenic effects of glyphosate in bone marrow cells of Swiss albino mice

Summary: Single doses of the glyphosate formulation, Roundup (>41% IPA salt), or controls, were administered intraperitoneally to two sets of four groups of 15, 10-12 week old, male Swiss albino mice. Group I, control animals, were given DMSO alone, Group II, positive control animals, were given B(a)P, and Group III and IV were given Roundup diluted in DMSO to 25 and 50 mg/kg bw of glyphosate respectively. Five animals from each group of each set were sacrificed at 24, 48 and 72 hours following treatment, and bone marrow was harvested from the animal's femurs.

In the first set, a total of 75 well spread metaphase plates per animal in each group was analyzed for chromosomal aberrations (breaks, fragments and exchanges) at a magnification of 100x and the mitotic index was calculated from a scan of 2000 cells per animal. The frequency of aberrant cells was significantly increased in Roundup-treated groups in a dose and time dependent manner with a reduction in mitotic index. At 72 hours, control, positive control, 25 mg/kg bw and 50 mg/kg bw Roundup-treated groups had the following percent incidences of aberrant cells: 1.74, 15.22, 7.76, and 9.24 and the following mitotic indices: 4.84, 1.94, 3.75, and 3.06 respectively.

In the second set, the frequency of micronucleated polychromatic erythrocytes (MNPCE) was evaluated. A minimum of 2000 erythrocytes was scored for each group. A significant increase in the frequency of MNPCE's was noted. At 72 hours, control, positive control, 25 mg/kg bw and 50 mg/kg bw Roundup-treated groups had the following frequency of MNPCE's/1000 PCE's: 1.18, 18.25, 6.12, and 8.48, respectively.

The results indicate that the glyphosate formulation, Roundup is clastogenic and cytotoxic to mouse bone marrow.

Limitations:

- A commercial formulation of glyphosate, Roundup (glyphosate as the isopropylamine salt >41%), was used rather than the active ingredient. Inert ingredients were not indicated.

- The method of dosing, via i.p. injection, is not a mode of dosing relevant to human health.

Conclusions: This study does not meet the minimum criteria to be eligible for risk assessment of the active ingredient, as outlined in the draft "Guidance for considering and using open literature studies to support human health risk assessment" (US EPA, 2011). The major limitations, as discussed above include the use of a commercial formulation rather the active ingredient, and the use of i.p. injection for dosing. This study is categorized as 'invalid' (inappropriate for quantitative or qualitative use in risk assessment).

Reference: Prasad, S., Srivasta, S., Singh, M. & Shukla, Y. (2009) Clastogenic effects of glyphosate in bone marrow cells of Swiss albino mice. J. Toxicol. 2009: Article ID 308985, 6 pages.

Study Title: (33) Evaluation of genome damage and its relation to oxidative stress induced by glyphosate in human lymphocytes in vitro

Summary*:* Technical grade glyphosate (98%) at concentrations of 0.5, 2.91, 3.5, 92.8 and 580 µg/ml was incubated with human blood for 4 hours at 37ºC, with and without metabolic activation (S9 from human liver). The first three concentrations correspond to levels of glyphosate likely to be encountered in residential and occupational exposure. The genotoxic and oxidative potential of glyphosate on human lymphocytes was then analysed in a series of assays. Ferric-reducing ability of plasma (FRAP), thiobarbituric acid reactive substances (TBARS) and the hOGG1 modified comet assay were used to measure glyphosate's oxidative potential and its impact on DNA. Genotoxicity was evaluated with the alkaline comet assay and by analysis of micronuclei and other nuclear instabilities.

The percentage of viable and nonviable lymphocytes in samples treated with glyphosate followed a linear response. Without the addition of S9, a significant increase in the number of early apoptotic and necrotic cells was detected only at 580 µg/ml; however, in the presence of S9 an increase in the number of apoptotic cells was noted at 2.91 µg/ml and above, although a significant increase in necrotic cells occurred only at 580 µg/ml.

Both the alkaline and hOGG1 comet assay indicated that glyphosate caused limited DNA damage in treated lymphocytes. In the alkaline comet assay, without S9, there was a significant increase in tail length (20.39 µm) at 580 µg/ml, and an increase in tail intensity (1.80%) at 3.5 µg/ml and above, compared to control values of 18.15 µm for tail length and 1.14% for tail intensity. With S9, tail length was significantly increased at 3.5 µg/ml and above and tail intensity was significant at the highest dose. With the hOGG1 comet assay, induction of oxidative damage did not occur in a clear dose-response relationship, but rather a significant increase in tail length was noted at 580 µg/ml without S9, and a single significant effect of increased tail intensity was noted only at 3.50 µg/ml with S9.

Without S9, the number of micronuclei (MN), nuclear buds (NB) and nucleoplasmic bridges (NPB) increased slightly at 3.5 µg/ml and above, however, only the increased numbers of NB's at 580 µg/ml was statistically significant. With the addition of S9 an increase was observed for all concentrations but it was significant for MN, NB and NPB only at 580 µg/ml.

Significantly increased FRAP values at 580 µg/ml with or without metabolic activation indicated an increased plasma antioxidant capacity. TBARS also increased significantly at 580 µg/ml with or without metabolic activation indicating an increase in lipid peroxidation.

The results suggest that glyphosate may cause oxidative damage to DNA, but statistical significance with various methods generally occurred only at the highest dose tested (580 µg/ml). Concentrations relevant to human exposure may not pose a significant health risk.

Limitations:

- The study does not follow a standard test guideline. The study involves a series of assays of glyphosate effects on blood cells obtained from human volunteers.
- The authors concede that the lack of statistical significance at lower concentrations may be due to the low number of samples included in the study.

Conclusions: This study does not meet the minimum criteria to be eligible for risk assessment of the active ingredient, as outlined in the draft "Guidance for considering and using open literature studies to support human health risk assessment" (US EPA, 2011). The major limitations, as discussed above

include the lack of a test guideline and the low number of samples in the study. This study is categorized as 'invalid' (inappropriate for quantitative or qualitative use in risk assessment).

Reference: Mladinic, M., Berend, S., Vrdoljak, A.L., Kopjar, N. Radic, B. & Zeljezic, D. (2009) Evaluation of genome damage and its relation to oxidative stress induced by glyphosate in human lymphocytes in vitro. Environ. Mol. Mutagen. 50(9): 800-807.

Study Title: (34) Time- and dose-dependent effects of Roundup on human embryonic and placental cells

Summary: The toxicity and endocrine disruption potential of glyphosate and Roundup were tested on human embryonic 293 cells, placental-derived JEG3 cells and other tissue.

The median lethal dose (LD_{50}) of Roundup in the 293 cell line was 0.3% (6.35 mM glyphosate) after 1 hour in serum-free medium and 0.06% (1.27 mM glyphosate) after 72 hours in the presence of serum. Comparable results were obtained with the slightly less sensitive JEG3 cell line. In all instances, Roundup (generally used in agriculture at 1-2%, i.e., with 21-42 mM glyphosate) was more efficient than glyphosate alone, suggesting an additional effect due to the adjuvants. The addition of serum (10% fetal calf serum) to the media buffered and delayed the toxic effect. Serum-free cultures revealed effects in the short-term (1 hour) that were visible 1-2 days later when serum was included in the media.

Non-toxic concentrations Roundup or glyphosate alone were able to act as aromatase activity inhibitors. This was shown after 24 hours in 293 cells transfected with the aromatase cDNA and also after 15 minutes in fresh placental cellular extracts (microsomal fraction). The IC_{50} was lowered from 2.4% glyphosate after 15 minutes in microsomes to 0.8% after 24 hours in 293 cells. Roundup was found to be an active inhibitor of aromatase activity in a variety of tissue and species (cell lines from placenta or embryonic kidney, equine testicular, or human fresh placental extracts). The inhibiting effect was dose and temperature dependent. Roundup showed greater inhibition than glyphosate alone.

The cytotoxic and potentially endocrine-disrupting effects of Roundup and glyphosate indicate a potential for adverse effects on human health. The study suggests that the tendency for regulatory agencies to examine pure substances rather than formulated mixtures may lead to an underestimation of the toxic or hormonal impact of a formulated mixture.

Limitations:

- The study does not follow a standard test guideline. The study involves testing the toxicity and endocrine disruption potential of glyphosate/Roundup on human embryonic and placental cells as well as other tissues. It is difficult to apply *in vitro* studies to *in vivo* human health risk.

- Percentage purity of the reagent grade glyphosate is not given.

Conclusions: This study does not meet the minimum criteria to be eligible for risk assessment of the active ingredient, as outlined in the draft "Guidance for considering and using open literature studies to support human health risk assessment" (US EPA, 2011). The major limitations is the lack of a standard test guideline. This study is categorized as 'invalid' (inappropriate for quantitative or qualitative use in risk assessment because it is of insufficient quality and lacks scientific defensibility).

Reference: Benachour, N., Sipahutar, H., Moslemi, S., Gasnier, C., Travert, C., & Séralini, G.E. (2007) Time- and dose-dependent effects of Roundup on human embryonic and placental cells. 53(1): 126-133.

Study Title: (35) Exposure to pesticides increases levels of uPA and uPAR in pre-malignant human prostate cells

Summary: Commercial herbicides (Glyphosate, Liberty), insecticides (Asana XL, Lorsban, Warrior), and selected active ingredients (glyphosate, chlorpyrifos, λ-cyhalothrin) were tested for their effect on the expression of urokinase (uPA) and urokinase receptor (uPAR) in a transformed but non-tumorigenic human prostate epithelial line, PZ-7. Cultures were treated with each chemical for one day, then lysed, immunoblotted and probed with a monoclonal uPAR antibody recognizing the native uPAR protein or a monoclonal anti-uPA.

Roundup and the insecticides Lorsban and Warrior induced significant uPA protein expression; Lorsban and Warrior also significantly increased expression of uPAR. Combinations of Roundup and either Lorsban or Warrior led to even greater induction of both uPA and uPAR protein levels than single agents alone. None of the active agents alone significantly affected uPA expression in the absence of the formulations chemicals. Cyhalothrin and chlorpyrifos treatments did increase levels of uPAR protein but this increase did not match levels seen with the whole pesticides and were not considered significant.

The results suggest that the active ingredients in pesticides are not solely responsible for their effects on uPA and uPAR, and the complete pesticide formulations can increase uPA and uPAR expression.

Limitations:

- The study does not follow a standard test guideline. The study involves testing a number of pesticides, including Roundup and glyphosate, for their ability to promote the expression of a protease system (uPA/uPAR) associated with prostate cancer in a in a transformed but non-tumorigenic human prostate epithelial culture. It is difficult to apply *in vitro* studies to *in vivo* human health risk. Glyphosate is neither mutagenic or oncogenic.

- Percentage purity of the reagent grade glyphosate is not stated; purity of the glyphosate in Roundup and the identity of the additional formulation ingredients in Roundup is not stated.

Conclusions: This study does not meet the minimum criteria to be eligible for risk assessment of the active ingredient, as outlined in the draft "Guidance for considering and using open literature studies to support human health risk assessment" (US EPA, 2011). The major limitations, as discussed above include the lack of a test guideline and no statement as to glyphosate purity in the study. This study is categorized as 'invalid' (inappropriate for quantitative or qualitative use in risk assessment).

Reference: Potti, A. & Seghal, I. (2005) Exposure to pesticides increases levels of uPA and uPAR in pre-malignant human prostate cells. Environ. Toxicol. Pharmacol. 19(2): 215-219.

Study Title: (36) Oral bioavailability of glyphosate: studies using two intestinal cell lines

Summary: The effect of glyphosate exposure on the barrier function of intestinal epithelium was examined using two polarized cell lines, a human colonic cell line with enterocytic differentiation (Caco-2), and a rat small intestinal crypt cell line (IEC-18) formed into monolayers on Transwell inserts. The apical side of the membranes was exposed to glyphosate and the permeability of electrolytes [transepithelial electrical resistance (TEER)], transport of [^{3}H]-mannitol, lactose dehydrogenase (LDH) release, and F-actin arrangements were determined.

TEER determinations across the monolayer measure electricophysiological properties and indicate paracellular permeability of the monolayer. In Caco-2 cells, glyphosate at either 10 or 20 mg/ml caused a significant dose-dependent decrease in TEER after 1 hours. After 4 hours, the 20 mg/ml dose had caused a drop of 96%. A smaller overall decline was noted with IEC-18 cells.

Transport of mannitol is also an indiction of paracellular permeability. The IEC-18 line was found to be 5-fold more permeable to mannitol than the Caco-2 line initially, and was not as affected by glyphosate. In the Caco-2 line, however, mannitol permeability was increased significantly above the control (3.5-fold) at a glyphosate concentration of 20 mg/ml.

Radiolabeled glyphosate was added to the apical side of the membrane and the amount detected in the basolateral side was measured over time. When the Caco-2 monolayer was exposed to 5 mg/ml glyphosate on the apical side more than 90% of glyphosate activity remained on the apical side and less than 2% was found in the basal chamber. When the highest concentration (20 mg/ml) of glyphosate was applied for 5 hours, approximately 8% had moved into the basal chamber. The rate of glyphosate transport was much higher in the IEC-18 line and was also concentration dependent; however, higher concentrations of glyphosate produced significant damage to the membrane. With the Caco-2 cells, the amount of labelled glyphosate retained on the membrane was less than 1% at all concentrations, with saturation of binding sites at 5 mg/ml and above. The IEC-18 cells were able to bind approximately 10-fold less glyphosate than the Caco-2 cells.

The effect of glyphosate on cell integrity was determined by LDH release. The amount of LDH which had leaked into the apical chamber was determined after incubation with glyphosate for 4 hours. A statistically significant increase in LDH leakage occurred at the two highest doses of glyphosate in the Caco-2 cells.

Tight junctions in gut epithelial cells regulate the movement of solutes and are connected to the actin cytoskeleton. Glyphosate was found to alter the staining pattern of F-actin fibers. Changes included loss of microvilli bundles, disruption of cell shape, diffuse junctions and disorganization and clumping of fibers.

The study shows that at low doses, glyphosate is able to penetrate the epithelial barrier only slightly. At higher doses, however, there are signs of damage to the epithelial cells leading to loss of paracellular barrier function at the tight junctions. At doses greater than 10 mg/ml, glyphosate significantly disrupts the barrier properties of cultured intestinal cells and is able to cross the epithelial membrane.

Limitations:

- The study does not follow a standard test guideline. The study involves testing the effect of glyphosate on the intestinal epithelial barrier using two cell lines. The applicability to *in vivo* human risk is unclear.

- Percentage purity of the reagent grade glyphosate is given as approximately 95% pure.

Conclusions: This study does not meet the minimum criteria to be eligible for risk assessment of the active ingredient, as outlined in the draft "Guidance for considering and using open literature studies to support human health risk assessment" (US EPA, 2011). The major limitations is the lack of a standard test guideline. This study is categorized as 'invalid' (inappropriate for quantitative or qualitative use in risk assessment).

Reference: Vasiluk, L., Pinto, L.J. & Moore, M.M. (2005) Oral bioavailability of glyphosate: studies using two intestinal cell lines. Environ. Toxicol. Chem. 24(1):153-160.

Study Title: (37) Cysteine turnover in human cell lines is influenced by glyphosate

Summary: HeLa and hepatoma human cell cultures were exposed to low doses of three chemically unrelated pesticides, bentazon at 150 and 1500 µg/L, metalaxyl at 100 µg/L and glyphosate at 100 and 1000 µg/L, for 24 hours. Total cysteine and glutathione concentrations were then assayed in the cells and in the media.

Extracellular cysteine levels were elevated in the presence of glyphosate in both cell cultures. Cysteine levels were similar at both glyphosate concentrations. Cysteine was also found to decrease in intracellular concentration in the hepatoma cell culture in the presence of glyphosate. There was no change in intracellular concentration of glutathione in either cell culture in the presence of glyphosate. No significant effect on intracellular or extracellular concentrations of cysteine or glutathione was found with bentazon or metalaxyl.

The results suggest that exposure to glyphosate can have a significant effect on the concentrations of intra- and extracellular cysteine. Cysteine is considered to be the limiting substrate for the synthesis of glutathione which is an important antioxidant. The effects noted are in the presence of micromolar concentrations of glyphosate which compare to concentrations observed in monitoring programs.

Limitations:

- The study does not follow a standard test guideline. The study involves testing a number of pesticides, including glyphosate, for their ability to effect cysteine and glutathione turnover in human cell lines.

- Percentage purity of the reagent grade glyphosate is not stated; number of glyphosate concentrations (2) was limited.

Conclusions: This study does not meet the minimum criteria to be eligible for risk assessment of the active ingredient, as outlined in the draft "Guidance for considering and using open literature studies to support human health risk assessment" (US EPA, 2011). The major limitations, as discussed above include the lack of a test guideline and no statement as to glyphosate purity in the study. This study is categorized as 'invalid' (inappropriate for quantitative or qualitative use in risk assessment).

Reference: Hultberg, M. (2007) Cysteine turnover in human cell lines is influenced by glyphosate. Environ. Toxicol. Pharmacol. 24(1):19-22.

August 29, 2012

Note To/Note au: Toxicology File

From/De: 2032
Evaluation Officer, TRS-2, HED

Subject/Objet: **Sub. No(s):** 2004-0175
Product Name: Glyphosate products
Active Ingredient: Glyphosate acid and salt forms (GPS)

Action Requested : **Screen of Open Literature Studies**

Study Title: The Hemodynamic Effects of the Formulation of Glyphosate-Surfactant Herbicides

Summary: In human incidences of poisoning with glyphosate-surfactant herbicides (GlySH), systemic toxicity is associated with gastrointestinal injury, laryngeal injury, pulmonary toxicity, impaired renal and liver function, leukocytosis, impaired neurological function, dermatitis, metabolic acidosis, arrhythmias, myocardial depression, shock and even death. Aspiration pneumonitis and upper respiratory tract irritation are also commonly reported findings.

Glyphosate has been proposed to play a role in uncoupling of mitochondrial oxidative phosphorylation in rat liver mitochondria. Evidence form studies suggested that glyphosate (isopropylamine salt form) is an uncoupler of electron transfer chain and may be able to act both as a chelator and mild protonophore (glyphosate increased permeability of mitochondrial membrane to protons and Ca^{2+}). Furthermore glyphosate had inhibitatory effect on energy-dependent transhydrogenase reaction *in vitro* and decreased the hepatic level of cytochrome P450 and monoxygenase activity (liver) and aryl hydrocarbon hydroxylase (intestines) *in vivo*. Mitochondria might be a critical target of toxic mechanism of GlySH, however the clinical significance between the mitochondrial effects and systemic toxicity is unclear.

In a retrospective study, data collected from 69 men and 62 women was analysed. Fatality of glyphosate poisoning was 8.4% (11 fatalities). The estimated amount of GlySH ingested and age averages were 122±12 mL/47±2 years among survivors and 330±42 mL/60±4 years among fatalities. Nausea with or without vomiting, sore throat and fever were the most common manifestations of GlySH poisoning. The most common laboratory abnormalities included leukocytosis, low bicarbonate, hepatic dysfunction, hypercapnea, acidosis, and renal insufficiency. Abnormal findings, most frequently sinus tachycardia and nonspecific ST-T changes, were observed in 15/81 electrocardiograms. Major prognostic predictors for fatality included shock, respiratory distress necessitating intubation, pulmonary edema, dysrhythmia, renal dysfunction necessitating hemodialysis, altered consciousness, and pulmonary edema.

Cardiovascular Toxicity:

Cardiovascular symptoms, noted in literature, of GlySH poisoning in humans include ECG abnormalities such as sinus tachycardia, sinus bradycardia, first degree AV block, as well as shock (possibly due to intravascular hypovolemia). In a beagle dog study, cardiac depression was observed with Roundup treatment and surfactant injection, suggesting suppression of the cardiac conduction system and contractility rather than intravascular hypovolemia plays a role in the shock induced by GlySH acute poisoning. In a mongrel dog study, an isopropylmaine glyphosate salt (IPA) injection showed positive, dose- dependent inotropic and chronotropic responses with increasing myocardial contraction, arterial pressure, and pulse pressure, as well as reduced vascular resistance in the hind leg.

Study:

A study was designed to characterize the major components leading to cardiovascular failure in GlySH poisoning. Male Landrance piglets (6-8 weeks old, 8-15 kg) were sedated (im) with ketamine and atropine and dosed with propofol and pancuronium bromide via percutaneous venous cannula in the marginal vein of the pinna. Piglets were intubated with endotracheal tubes, ventilated (12 breaths/min), provided saline with 5% glucose (dripping 5mL/kg/hr via marginal vein iv) and regularly paralyzed with iv pencuronim (maintained with 2-3% isoflurane) while physiological variables were monitored (for e.g. blood pressure -BP, mean arterial blood pressure -MABP, heart beats and electrocardiography- ECG and blood sampling). Five experimental groups (6 animals/group) were: 1) control with saline; 2) glyphosate 360 mg/mL in NaOH; 3) IPA 126 mg/mL in water; 4) glycine monoisipropylamine salt solution, IPAG, 40% wt; POEA, 15% in water.

In a preliminary study, most piglets died soon after IPAG infusion at rate higher than 10mL/h. Sudden death might have been caused by a reduction in BP with ventricular arrhythmia, or the high infusion rate could cause a reversible depression of left-ventricular function after discontinuing infusion right after MABP decreased to 50% of initial value. For other chemicals, no obvious reductions in MABP were noted within one hour infusion at the same rate. Piglets were therefore infused with IPAG at 10 mL/hr rate but 50% reduction in MABP from initial value was chosen as endpoint, with survivors observed for up to 2 hours from the initialization of dosing. Piglets in other treatment groups were infused at the same rate for 1 hour and observed for one hour after cessation of doing. Daily activities and urine amounts were recorded for two days, while blood sampling was performed at 24 and 48 hours after the dosing.

The average infused dose of IPAG, glyphosate, IPA and POEA was 159.8±15.79 mg/kg bw, 238.47±17.49mg/kg bw, 75.24±4.51mg/kg bw, 0.0944±0.00546 mg/kg bw, respectively. Both POEA and IPAG had a fatality rate of 4/6 (66.7%). Results are summarized below:

IPA: elevated MABP and pulmonary vascular resistance index (PVRI) and reversible right-ventricular stroke work index (RSWI).

IPAG: high death rate (66.7%); lowered cardiac contractility and MABP with increases in mean pulmonary artery pressure (MPAP) and vascular resistance (leading to heart failure); blood lactate formation with lowered (base excess BE) (with 50% of the dose, in the concentration similar to other chemicals); decreased urine volume 24 and 48 hours after. Since acidosis or alkalosis, pulmonary rales, and hypoxemia were not observed, the noted changes were attributed to direct depressive cardiovascular and vasoactive effects exerted by IPAG instead of to uncoupling mitochondrial oxidative phosphorylation and reduced respiratory control ratios of mitochondria (which would explain lactate formation and acidosis).

POEA: progressively decreased left-side ventricular function (decreased cardiac index- CI, left ventricular stroke work index- LVSWI and increased pulmonary capillary wedge pressure- PCWP and central venous pressure- CVP), decreased BE, increased pulmonary vasoconstriction effects (increased PCWP and CVP), increased pulmonary vasoconstriction effects (increased MPAP and PVRI) leading to metabolic acidosis (decreased pH) and increased lactate (likely due to circulatory collapse); 66.7% mortality (between 1-3 hours post dosing, possibly from uncorrected metabolic acidosis); decreased urine volume 24 and 48 hours after.

Glyphosate: reduction in pH and BE without significant hemodynamic changes.

Study demonstrated that negative cardiovascular effects seen with GlySH poisoning could be attributed to POEA, IPAG or both; and showed that adjuvants can be toxic.

Limitations:

- The test substance was a glyphosate based commercial formulation. The percent active ingredient was not indicated. Other components of the formulation were not identified besides the glyphosate and isopropylamine salt of glyphosate. The remaining components were identified as inert ingredients. Volume of ingestion was approximated and often self-reported.

- several patients had co-ingestants, including sedative drugs (2), hypnotics (3), wine (3) and paraquat (1)

- pigs are atypical animals used in toxicology studies reviewed by regulatory agencies

- purities of test substances were not specified, and only a single dose of each test substance was utilized.

Conclusion: This study does not meet the minimum criteria to be eligible for risk assessment of the active ingredient, as outlined in the draft "Guidance for considering and using open literature studies to support human health risk assessment" (US EPA, 2011). The major limitations included not identifying the purity of the test substances and inadequate number of dose levels. This study is categorized as 'invalid' (inappropriate for quantitative or qualitative use in risk assessment because it is of insufficient quality and lacks scientific defensibility).

Reference: Hsin-Ling Lee and How-Ran Guo (2011). The Hemodynamic Effects of the Formulation of Glyphosate-Surfactant Herbicides, Herbicides, Theory and Applications, Prof. Marcelo Larramendy (Ed.), ISBN: 978-953-307-975-2, InTech, Available from: http://www.intechopen.com/books/herbicides-theory-and-applications/thehemodynamic-effects-of-the-formulation-of-glyphosate-surfactant-herbicide.pdf

Oct 16, 2012

Note To/Note au: Toxicology File

From/De: 2032
Evaluation Officer, TRS-2, HED

Subject/Objet: **Sub. No(s):** 2004-0175
Product Name: Glyphosate products
Active Ingredient: Glyphosate acid and salt forms (GPS)

Action Requested : Screen of Open Literature Studies

Study Title: Genotoxicity of AMPA, the environmental metabolite of glyphosate, assessed by the Comet assay and cytogenetic tests.

Summary: AMPA (99%), the major metabolite of glyphosate was evaluated for genotoxic potential, *in vitro*, using the Comet assay in Hep-2 cells and chromosome aberration (CA) test in human lymphocytes, and *in vivo* through micronucleus test in mice for assessing chromosomal damage.

In the Comet assay, Hep-2 cells were incubated for 4 hours with 2.5, 4.5, 5.5, 6.5, 7.5, 9 or 10.0mM AMPA. Mitomycin C (0.01 mM) and medium were used as positive and negative controls, respectively. Tail moment (TM), DNA percentage in tail (% of DNA) and tail length (TL) were used to estimate DNA damage. Concentration above 7.5 mM AMPA were not used for nontoxicity analysis, due to <80% viability. AMPA exhibited dose-dependent genotoxic effect in all three parameters, increasing extent of DNA migration.

In a CA test, 0.9 or 1.8mM AMPA was tested on lymphocytes from 6 different human blood samples with 48 hr treatment. A significant increase ($p<0.05$) in the number of CA, including gaps, was observed after 1.8mM AMPA (9.5±1.1 CA/100 metaphases, a two fold increase over control), with most aberrations being of chromatid type.

In a micronucleus test (MNT), male and female Balb-C mice received i.p. injections of 100 or 200 mg/kg AMPA, repeated once after 24 hours. Saline and a single injection of cyclophosphamide injection of 20 mg/kg were used as controls. Animals were sacrificed 24 hours post second treatment and 10±1.9 and 10.4±3.3 micronucleated erythrocytes (MNE)/1000 analyzed cells were observed with 100 or 200 mg/kg AMPA, respectively, versus a basal level of 3.8±1.8 (negative control).

AMPA was found to be genotoxic in Comet assay and chromosomal aberration *in vitro* and in micronucleus test in mice *in vivo*, however mixed results and scarce data is available for AMPA potential genotoxicity in literature.

Limitations:

- Test substance was the primary metabolite of glyphosate, not glyphosate itself.

Conclusion: This study does not meet the minimum criteria to be eligible for risk assessment of the active ingredient, as outlined in the draft "Guidance for considering and using open literature studies to support human health risk assessment" (US EPA, 2011). The major limitation as discussed above was the use of AMPA not glyphosate. This study is categorized as 'invalid' (inappropriate for quantitative or qualitative use in risk assessment because it is of insufficient quality and lacks scientific defensibility).

Reference: F.Manas, L.Peralta, J.Raviolo, H.Garcıa Ovando, A.Weyers, L.Ugnia, M. GonzalezCid, I.Larripa, N.Gorla (2009). Genotoxicity of AMPA, the environmental metabolite of glyphosate, assessed by the Comet assay and cytogenetic tests. Ecotoxicology and Environmental Safety. 72: 834-837.

Oct. 3, 2012

Note To/Note au: Toxicology File

From/De: 2032
Evaluation Officer, TRS-2, HED

Subject/Objet: **Sub. No(s):** 2004-0175
Product Name: Glyphosate products
Active Ingredient: Glyphosate acid and salt forms (GPS)

Action Requested : **Screen of Open Literature Studies**

Study Title: Ethoxylated adjuvants of glyphosate-based herbicides are active principles of human cell toxicity.

Summary: Three cell lines, human embryonic kidney (HEK) 293 cell line, hepatoma cell line HepG2 and JEG3 cell line were used to test cytotoxicity of 9 glyphosate-based formulations *in vitro* for 24 hours, using polyethoxylated tallowamine POE-15 and Genamin, and glyphosate (G) alone as controls. The formulations included: Bayer GC (12.5% G, 1-5% of POE-15), Clinic EV (42% G, 11% of POE-15), Genami T200 (60-80% POE-15), Glyphogan (39-43% G, 13-18% of POE-15), Roundup Grand Travaux (400 g/L G), Roundup Grand Travaux plus (450 g/L G, 90 g/L ethoxylated etheralkylamine), Roundup Ultra (41.5% G, 16% surfactant), Roundup Bioforce (360g/L G, 1-5% of POE-15), Roundup 3plus (170g/L G, 8% surfactant homologation), Topglypho 360 (360/L G), POE-15.

Effects on the mitochondrial succinate dehydrogenase (SD) activity, reflecting cell respiration inhibition, were measured in serum free medium with glyphosate formulation and adjuvants and glyphosate alone after 24 hour exposure. All chemicals induced cell toxicity, with similar dose-dependent pattern on all three cell lines, although JEG3 cells were up to 2-fold more sensitive to treatments. The adjuvants POE 15 and Genamin alone were the most toxic (LC50 2-7 ppm), while glyphosate was the least toxic (1192-19323 ppm). The middle toxicity group (~100 times less toxic) included Roundup GT, Roundup GT+, Clinic EV, Top Glypho 360, Glyphogran and Bayer GC. The least toxic group (~100 times less toxic again) included Roundup Ultra, Bioforce and 3plus. Toxicity was determined to be dependent on concentration of ethoxylated adjuvants not glyphosate itself in the formulations, in a linear manner to some extent. Glyphosate did not buffer or amplify direct POE-15 toxicity.

Adjuvants were analyzed for composition using spectrum characteristic and showed four main spectra patterns; one centered around 900m/z, 600m/z, 500 m/z and 300 m/z (identified as POE-2). Ethoxylated adjuvants were considered as the active principle of the toxicity of glyphosate based herbicides in human cells. Furthermore, the mechanism of action of adjuvants was determined to be due to disruption of the cellular membranes by micellization with the critical micelle concentration beginning at 3ppm for POE-15. POE-15 and glyphosate formulations with ethoxylated adjuvants induced more necrosis by membrane alterations than apoptosis that was noted at higher levels with glyphosate.

Ethoxylated adjuvants were not considered inert, but cell membrane disruptors that induce severe mitochondrial alterations.

Limitations:

- Glyphosate purity was unspecified, and only a single concentration was used as a control; glyphosate formulations were used with incomplete content identification

- Actual data is mainly presented in graph format, making evaluation of the data difficult. Data for LC50 for glyphosate in HepG2 cell line was not determined.

- Since the study was *in vitro* and only for 24 hours, and it is not clear how the *in vitro* results would translate to *in vivo* toxicity

Conclusion: This study does not meet the minimum criteria to be eligible for risk assessment of the active ingredient glyphosate, as outlined in the draft "Guidance for considering and using open literature studies to support human health risk assessment" (US EPA, 2011). The major limitation was focus on adjuvants not the active ingredient, as well as deficiencies in reporting of study data. This study is categorized as 'invalid' (inappropriate for quantitative or qualitative use in risk assessment because it is of insufficient quality and lacks scientific defensibility).

Reference: Mesnage, R., Bernay, B., and Seralini, G.-E. (2012). Ethoxylated adjuvants of glyphosate-based herbicides are active principles of human cell toxicity. Toxicology XX (In press)

Sept 10, 2012

Note To/Note au: Toxicology File

From/De: 2032
Evaluation Officer, TRS-2, HED

Subject/Objet: **Sub. No(s):** 2004-0175
Product Name: Glyphosate products
Active Ingredient: Glyphosate acid and salt forms (GPS)

Action Requested : **Screen of Open Literature Studies**

Study Title: Comparative effects of the Roundup and glyphosate on mitochondrial oxidative phosphorylation. (#:)

Summary: Roundup and glyphosate effects on mitochondrial bioenergetics were studied in isolated rat mitochondria from starved (overnight) Wistar rats.

To measure respiratory rates, mitochondria (0.8mg) were incubated in 1mL of respiratory standard medium (25ºC) in the presence of glyphosate or Roundup equivalent to 0, 0.5, 1, 2, 5, 10 or 15 mM glyphosate. State 4 respiration was initiated by addition of succinate (10mM), and State 3 respiration was energized by succinate (10mM) and initiated by addition of ADP (1.5mM) 2 minutes after. Glyphosate did not affect the respiratory control ratio (RCR) and ADP to oxygen ration (ADP/O) up to 5mM, while Roundup significantly reduced both ratios. Roundup (up to 15mM) depressed Stage 3 and uncoupled respiration rates, and stimulated respiration for Stage 4 two-fold. Control with antimycin A indicated that oxygen consumption occurred exclusively due to respiratory activity.

Transmembrane potential was measured with succinate support in similar incubation and concentrations of glyphosate and Roundup. For further measurements, mitochondria were supplemented with TPP+ (3µM) and rotenone (2µM) for 5 min in presence of Roundup (0, 0.5, 1 or 3mM), energized with succinate (10mM) and with ADP (150µM) added to initiate phosphorylation. Valinomycin was added at the end of each assay to elicit completer collapse of membrane potential. Glyphosate did not affect the transmembrane potential (up to 15 mM), while Roundup almost collapsed the transmembrane potential (up to 10mM) that was promoted by succinate. In addition, Roundup not only decreased the depolarization and repolarization amplitude induced by ADP, but also lengthened the lag phase preceding repolarization (indicating inhibitory effect on phosphorylation efficiency).

Enzymatic activities of the respiration complexes II and IV of the mitochondrial respiratory chain (succinate dehydrogenase, succinate cytochrome c reductase and cytochrome c oxidase respectively) and ATPase and ATPase synthase were not affected by glyphosate (up to 15mM). Roundup, on the other hand, partially inhibited complex II and III, indicating that Roundup interacts with electron transfer at that level, but not the terminal segment of chain. Roundup also significantly depressed ATPase activity (60%) at up to 15mM and almost completely inhibited ATPase synthase activity (91%) at 5mM.

Induction of mitochondrial swelling in valiomycin (1µM)-treated mitochondria (0.3 mg), incubated in hyposmotic K^+-acetate (54 mM) medium was studied with glyphosate and Roundup. Neither Roundup nor glyphosate induced significant swelling. Mitochondrial permeability underwent a transition in Ca2+-loaded (50µM) mitochondria treated with ruthenium red (1µM) and Roundup but not with glyphosate.

In conclusion, glyphosate alone did not show any relevant effect on the mitochondrial bioenergetics, unlike Roundup. The so called "inert" unspecified ingredients in glyphosate formulation are likely responsible for the observed toxicity at the bioenergetics level.

Limitations:

- The test substance was a glyphosate and Roundup, glyphosate commercial formulation.

- The percent active ingredient was not indicated and purity of glyphosate was not specified. Other components of the formulation were not identified.

Conclusion: This study does not meet the minimum criteria to be eligible for risk assessment of the active ingredient, as outlined in the draft "Guidance for considering and using open literature studies to support human health risk assessment" (US EPA, 2011). The major limitations as discussed above were not characterizing the test substance properly. This study is categorized as 'invalid' (inappropriate for quantitative or qualitative use in risk assessment because it is of insufficient quality and lacks scientific defensibility).

Reference: Francisco Peixoto (2005). Comparative effects of the Roundup and glyphosate on mitochondrial oxidative phosphorylation. Chemosphere 61:1115-1122.

Sept 11, 2012

Note To/Note au: Toxicology File

From/De: 2032
Evaluation Officer, TRS-2, HED

Subject/Objet: **Sub. No(s):** 2004-0175
Product Name: Glyphosate products
Active Ingredient: Glyphosate acid and salt forms (GPS)

Action Requested : Screen of Open Literature Studies

Study Title: Comparison of the effect of Roundup Ultra 360 SL pesticide and its active compound glyphosate on human erythrocytes.

Summary: Roundup Ultra 360 L (360 g of isopropylamino salt/L) and glyphosate (95%) effects were studied on human erythrocytes and hemoglobin separated from whole blood of healthy Polish donors. Incubation of erythrocytes with glyphosate (doses of 100-1500 ppm) did not change the percentage of hemolysis after 1-5 h of incubation; however hemolysis was statistically significantly increased at highest dose after 24 hours of incubation (double the base value). Previously published data showed slight hemolysis of human erythrocytes at lower doses of Roundup Ultra 360 SL (1500 ppm after 1hour of incubation, and 500 ppm after 24 hours of incubation). Both glyphosate and Roundup Ultra 360 SL caused an increase in lipid peroxidation in erythrocytes after one hour of incubation at 1000 and 1500 ppm. Increased activity of catalase and increased oxidation of hemoglobin to methemoglobin were also noted after incubation with glyphosate and Roundup 360 SL for 1 hour (showing statistical significance at 1000 ppm and 1500 ppm doses). It is possible that the observed change in catalase activity was directly associated with methemoglobin production. Since a consequence of oxidative stress in cells maybe a decrease in the concentration of reduced glutathione and increase of its oxidized form (GSH/GSSG), the level of reduced glutathione was measured. There was no significant change in the levels of reduced glutathione after glyphosate and Roundup Ultra SL incubation, as compared to control, indicating low toxicity. Overall, Roundup Ultra 360 SL produced slightly higher changes in the function of erythrocytes than glyphosate alone, likely due to other formulation ingredients.

The lack of accumulation of glyphosate in the organism, and relatively low toxicity in human erythrocytes at doses which might potentially occur in the human body lessens the concern for glyphosate safety in human erythrocytes.

Limitations:

- Atypical protocol for toxicology study with pesticide due to human tissue in vitro, with poor description of blood donors
- *In vitro* effects are difficult to translate to *in vivo* effects
- Positive control was not included
- pH measurement was not included
- Other components of the formulation were not identified.

Conclusion: This study does not meet the minimum criteria to be eligible for risk assessment of the active ingredient, as outlined in the draft "Guidance for considering and using open literature studies to support human health risk assessment" (US EPA, 2011). The major limitation as discussed above was inadequate protocol data. This study is categorized as 'invalid' (inappropriate for quantitative or qualitative use in risk assessment because it is of insufficient quality and lacks scientific defensibility).

Reference: Pieniazek, Danuta, Burkowska, Bozena and Duda, Wirgiliusz (2004). Comparison of the effect of Roundup Ultra 360 SL pesticide and its active compound glyphosate on human erythrocytes. Pesticide Biochemistry and Physiology 79:58-63.

Sept 4, 2012

Note To/Note au: Toxicology File

From/De: 2032
Evaluation Officer, TRS-2, HED

Subject/Objet: **Sub. No(s):** 2004-0175
Product Name: Glyphosate products
Active Ingredient: Glyphosate acid and salt forms (GPS)

Action Requested : **Screen of Open Literature Studies**

Study Title: Genotoxicity testing of the herbicide Roundup and its active ingredient glyphosate isopropylamine using the mouse bone marrow micronucleous test, Salmonella mutagenicity test, and Allium anaphase-telophase test.

Summary:

Mouse bone marrow micronucleus test: NMRI-born mice (11 weeks old) were intraperitonealy injected (3-5 mice/sex/group) with glyphosate isopropylamine salt (100, 150 or 200 mg/kg bw), or Roundup (133 or 200 mg/kg bw calculated as glyphosate isopropylamine). Methyl methanesulfoante (MMS) was used as a positive control and 0.9% NaCl as a negative control. Cells from bone marrow of mice were harvested 24 and 48 hours later for glyphosate and 24 hours for Roundup. Frequency of micronucleated polychromatic erythrocytes (MNPCE) was determined by examining 1000 PCE/animal. No significant difference was noted among sexes. The number of PCE was statistically significantly decreased at the highest concentration of Roundup (equivalent to 200 mg/kg bw of glyphosate isopropylamine) only, indicating that Roundup is more toxic than glyphosate isopropylamine salt.

Salmonella mutagenicity test: *Salmonella typhimurium* strains TA98 and TA199 were used to test mutagenicity of Roundup with S9 mix with three plates per dose in two experiments. Positive controls used included 10µg 4- nitro-o-phenylenediamine (NPD), 0.5µg MMS, 1.0 µg benzo(a)pyrene (B(a)P), 0.1µg 2-nitrofluorene and 0.4 µg sodium azide/plate. A slight but significant number of revertants was observed at 360µg/plate for TA98 (-S9) and 720 µg/plate for TA100 (+S9), indicating that Roundup, at concentrations close to the level of cytotoxicity, can induce point mutations. No percent survival of cells was measured at different Roundup concentrations in this test, only assuming cytotoxicity at the 1081µg/plate and 1440µg/plate.

Allium anaphase-telophase test: *Allium cepa* onion bulbs were allowed to produce roots in test solutions (6/solution) with: negative control (water), glyphosate isopropylamine (720 µg/L, 1440 µg/L, 2880 µg/L), positive control MMS (10 µg/L), Roundup (equivalent to 720 µg/L, 1440 µg/L, 2880 µg/L of glyphosate isopropylamine) with treatment replenished daily for 5 days. On the second day, the onions with poorest growth/test solution were removed. The length of the root bundles were measured and averaged as a percent of a control. The growth inhibition value, EC50, was interpolated form a graph of root length (%) vs log of concentration of test solution. Approximately 100 anaphase/early telophase cells were analyzed per onion. A statistically significant increase in chromosome aberrations which included "other aberrations" (weak spindle disturbances) was noted at two highest concentrations of Roundup, however without dose response (highest dose had less aberrations than the mid-high dose). The aberrations caused by positive controls included bridges and fragments. Cytotoxicity test was not performed; however there was a decrease in the mitotic index at the highest concentration of Roundup indicating a toxic effect of the formulation. Comparison of EC50 (5.5 mg/L vs 1.2 mg/L) indicates that Roundup is approximately 5 times more toxic to onion root cells than glyphosate isorpopylamine salt.

The guidelines for the studies included procedures by Schmid (1975), Fiksesjo (1985), and Ames 1975).

Limitations:

- The purity of active ingredient was not indicated. Other components of the formulation were not identified.

- Cytotoxicity/cell survival was not measured.

- Not enough different bacterial strains were used

Conclusion: This study does not meet the minimum criteria to be eligible for risk assessment of the active ingredient, as outlined in the draft "Guidance for considering and using open literature studies to support human health risk assessment" (US EPA, 2011). The major limitations as discussed above were inadequate data collection, poor characterization of test substance and incomplete protocol planning. This study is categorized as 'invalid' (inappropriate for quantitative or qualitative use in risk assessment because it is of insufficient quality and lacks scientific defensibility). It is unclear which components of Roundup formulation lead to the reported toxicity.

Reference: Rank, J., Jensen, A.-G., Skov, B., Pendersen, L.H. and Jensen, K. (1993). Genotoxicity testing of the herbicide Roundup and its active ingredient glyphosate isopropylamine using the mouse bone marrow micronucleous test, Salmonella mutagenicity test, and Allium anaphase-telophase test. Mutation Research. 300:29-36.

Sept 17, 2012

Note To/Note au: Toxicology File

From/De: 2032
Evaluation Officer, TRS-2, HED

Subject/Objet: **Sub. No(s):** 2004-0175
Product Name: Glyphosate products
Active Ingredient: Glyphosate acid and salt forms (GPS)

Action Requested : **Screen of Open Literature Studies**

Study Title: Differential effects of glyphosate and Roundup on human placental cells and aromatase. (#:)

Summary: Glyphosate and Roundup (360 g/L) were tested on human placental JEG3 cells and evaluated as possible endocrine disruptors, including their effect on aromatase (a mammalian cytochrome P450 enzyme crucial for sex steroid hormone synthesis) present in microsomes from human placenta and equine testis.

Cell viability was tested at Roundup concentrations up to 2% (in water), after 18, 24 or 48-hour exposures in a serum containing medium, by the MTT assay, compared to glyphosate, both with adjusted pH. Toxicity increased with time; LC50 was approximately 1.8 times lower for Roundup than for glyphosate, and the difference was noticeable within an hour of treatment in serum-free medium. Acidity (if pH was not adjusted) of 2% solution of Roundup or glyphosate, reduced cell viability by 23% after 18 hours in another experiment, and thus only partially contributed to the 90% reduction of cell viability observed at this concentration. When 0.1% of Roundup was added to glyphosate, increasing the concentration adjuvants mainly, the cell viability was significantly diminished (indicating toxic effects of the adjuvants).

After one hour incubation of cells with Roundup, estrogen synthesis was enhanced by 40%. A clear inhibition of aromatase was noted after 18 hours, as shown by decreased aromatase mRNA, with an IC50 of 0.04% for Roundup. Glyphosate alone did not inhibit estrogen synthesis, but when minute amounts of Roundup were added to cultures, aromatase activity was inhibited. Aromatase was also inhibited by Roundup and glyphosate separately in human and equine microsomes, with IC 50 of 0.6%, and approximately 2.0%, respectively. Kinetic parameters were examined for Roundup, showing competitive inhibition. An experiment with purified enzyme moieties from aromatase-rich equine testis showed a direct interaction of glyphosate with the equine aromatase active site. Spectral interaction showed a type II spectrum, characteristic of an interaction between nitrogen atom of the herbicide and the heme iron of the cytochrome. In addition, electron donor reductase (NADPH-dependent) was also directly inhibited by Roundup (IC50 5%) but to a lesser extent than cytochrome 450 aromatase responsible for the steroid binding and catalysis.

In conclusion, Roundup was found to be overall more toxic than glyphosate and affected endocrine metabolism in mammals *in vitro.*

Limitations:

- The test substances were a glyphosate commercial formulation (Roundup) and pure technical glyphosate. The percentage of active ingredient in the formulation was not indicated and the purity of glyphosate was not specified. Other components of the formulation were not identified.

Conclusion: This study does not meet the minimum criteria to be eligible for risk assessment of the active ingredient, as outlined in the draft "Guidance for considering and using open literature studies to support human health risk assessment" (US EPA, 2011). The major limitations as discussed above were not characterizing the test substance properly, and experiments focusing more on the formulation not the active ingredient itself. This study is categorized as 'invalid' (inappropriate for quantitative or qualitative use in risk assessment because it is of insufficient quality and lacks scientific defensibility).

Reference: Richard, S., Moslemi, S., Sipahutar, H., Benachour, N. and Seralinin, G-E. (2005). Differential effects of glyphosate and Roundup on human placental cells and aromatase. Environmental Health Perspectives. 113(6): 716-720.

August 22, 2012

Note To/Note au: Toxicology File

From/De: 2032
Evaluation Officer, TRS-2, HED

Subject/Objet: **Sub. No(s):** 2004-0175
Product Name: Glyphosate products
Active Ingredient: Glyphosate acid and salt forms (GPS)

Action Requested : Screen of Open Literature Studies

Study Title: Glyphosate and AMPA in drinking water

Summary: Glyphosate (CAS No. 1071-83-6) is a broad-spectrum herbicide with a major metabolite being aminomethylphosphonic acid (AMPA) (CAS No. 1066-51-9). Glyphosate is chemically stable in water and is not subject to photochemical degradation.

Absorption of a single dose of radiolabelled glyphosate from gastrointestinal tract was incomplete (~<30% dose) in rats and rabbits. By day 7, glyphosate was widely distributed throughout the rat body, with the highest concentration noted in bones. Biotransformation inside the body was minimal, with excretion mostly as a parent compound via urine and feces (rat), and only 0.2-0.3% as AMPA. AMPA was moderately absorbed (20%) in rats, and excreted almost exclusively via urine, and >0.1% as expired carbon dioxide.

Glyphosate and AMPA have similar chemical structure and toxicological profiles, and both are considered to exhibit low toxicity.

Glyphosate:
Glyphosate has very low acute toxicity by the oral and dermal routes (1950 to>5000 mg/kg bw) in rats, mice and goats.

Short term exposure with glyphosate technical had no effect on appearance or survival, but growth retardation and increased weights of brain, heart and kidneys were noted in one rat study (NOAEL of 1890 mg/kg bw/day). Limited histopathology showed no adverse effects. When lower doses were used in another study (NOAEL of 507 mg/kg bw/day), haematology, blood chemistry, urinalysis, and organ weights were unaffected. In yet another rat study, clinical chemistry was affected, showing increased alkaline phosphate and alanine aminotransferase (410 /852.5 mg/kg bw/day ♂/♀). In addition, decrease in sperm count (>1640 mg/kg bw/day) and cytoplasmic alterations in the parotid and submandibular salivary glands (basophilic changes and hypertrophy of acinar cells at 205/213 mg/kg bw/day ♂/♀, no NOAEL) were observed. In mice, reduced weight gains (50 000 mg/kg) and dose-dependent lesions in the parotid gland were observed at higher doses only (6250 mg/kg bw/day; NOAEL of 3125 mg/kg bw/day). No adverse effects were noted in a dog study (capsule) (NOAEL of 500 mg/kg bw/day).

In a combined chronic toxicology/carcinogenicity study in mice, no effect on survival or appearance and no increased incidence of neoplastic lesions were noted (NOAEL of 814 mg.kg bw/day). High dose males had decreased body weights and increased incidence of central lobular hepatocyte hypertrophy and hepatocyte necrosis. Mid-and high dose males also had hyperplasia of the urinary bladder. In a rat chronic study, no effect was noted on survival, appearance, haematology, blood biochemistry, urinalysis and organ weights. A statistically significant increase was noted in incidence of interstitial cell tumours

in rat testes (NOAEL 32 mg/kg bw/day), however similar findings were not found in another chronic rat study with higher doses. In the second rat study, liver weights were increased in high dose males, and increased incidence of inflammation of the gastric squamous mucosa was observed in the mid- and high dose animals (NAOEL of 410 mg/kg bw/day). Increased incidence of pancreatic islet cell adenomas (low and high-dose) and degenerative lens changes (high dose males) were within historical control. Glyphosate was not genotoxic in a range of *in vitro* and *in vivo* assays.

In a developmental rat study (gavage), increased incidence of soft stools, diarrhea, breathing rattles, red nasal discharge, reduced activity, increased mortality (6/25 dams), growth retardation, increased incidence of early resorptions, decreases in total number of implantations and the number of viable fetuses, and increased number of fetuses with reduced ossification of sternebra were observes at the high dose (NOAEL of 410 mg/kg bw/day). Similar effects were noted in rabbits, with presence of diarrhea and soft stools, mortality (2, 10) starting at mid-dose, and incidence of nasal discharge at high dose (NOAEL of 175 mg/kg bw/day). In a three-generation rat study (diet), only an increased incidence of unilateral renal tubular dilation was noted in F3b high dose male pups (NOAEL 30 mg/kg bw/day). In a more recent two-generation rat study, soft stools and decreased parental body weights were observed. In addition, slightly decreased litter size and pup weights were noted starting at the mid-dose.

AMPA:

AMPA is slightly hazardous to rats (LD50 of 8300 mg/kg bw). No dermal or ocular irritation was noted in rabbits.

In a short term toxicity study with rats, a decrease in body weight was noted at mid dose in males and high dose males and females. A significant increase in lactate dehydrogenase activity was noted starting at mid- dose, and increased aspartate aminotransferase activity and cholesterol levels were noted at the high dose. Additionally, a significant decrease in urinary pH and increased amounts of calcium oxalate crystals were observed in urine of high dose animals, and dose-related irritation of the mucosal and submucosal layers of the urinary tract starting at the mid-dose (more pronounced in males), and epithelial hyperplasia in the renal pelvis at the highest dose (NOAEL of 400 mg/kg bw/day). No treatment effects were observed in a short term study in dogs (NOAEL of 300 mg/kg bw/day), except at higher doses in a range finding study where (not reproduced) decreased hemoglobin, packed cell volume and erythrocyte counts were affected in couple of animals only.

No genotoxic activity was noted in studies with AMPA in bacteria and mammalian cells *in vitro*, and micronucleus formation *in vivo;* however no assays for gene mutation were performed in mammalian cells *in vitro.* Because of similarity of AMPA to glyphosate, positive results for gene mutation were not expected.

In a developmental rat study, dose-related increases in incidences of soft stools, mucoid feces and hair loss were noted starting at the mid-dose. Short periods of decreased body weight gain and food consumption were noted at high dose. Fetal body weight was decreased at the high dose, but no teratogenic effects were observed (developmental NOAEL of 400 mg/kg bw/day). No rabbit study was summarized.

No chronic/oncogenicity study of AMPA was carried out. One glyphosate study was contaminated with AMPA (0.61%), leading to NOAEL of 2.7 mg/kg bw/day of AMPA; however findings of decreased body weight gain in females and increased incidence of degenerative lenticular changes in males at the high dose, were likely mainly due to glyphosate, so the derived AMPA NOAEL is not likely reliable, according to this reviewer. No evidence of carcinogenicity was noted in that study. Similarly, no reproductive study with AMPA was been carried out. In a glyphosate + AMPA study, NOAEL for

AMPA was 4.5 mg/kg bw/day, but most of the effects were likely caused by glyphosate which was present in much larger percentage.

AMPA was not considered to be of greater toxicological concern than glyphosate, its parent compound. JMPR established ADI for AMAPA (alone or with glyphosate) to be 0.3 mg/kg bw based on NOAEL of 32 mg/kg bw/day (chronic rat study and UF of 100x). A health-based value of 0.9 mg/litre can be derived based on the group ADI (assuming 60kg adult drinking 2L/day, 10% of ADI to consumed water). Because of their low toxicity, the health-based value derived for AMPA alone or in combination with glyphosate is orders of magnitude higher than concentrations of glyphosate or AMPA normally found in drinking-water. Under usual conditions, therefore, the presence of glyphosate and AMPA in drinking-water does not represent a hazard to human health. For this reason, the establishment of a guideline value for glyphosate and AMPA is not deemed necessary.

Limitations:

- Only summaries of studies were included. Reproductive studies with glyphosate included only limited histopathological examination.

- Long term studies and reproductive toxicity studies for AMPA are unavailable.

Conclusion: This publication does not meet the minimum criteria to be eligible for risk assessment of the active ingredient, as outlined in the draft "Guidance for considering and using open literature studies to support human health risk assessment" (US EPA, 2011). The major limitation as discussed above was that it was a review with limited reporting of data. This study is categorized as 'invalid' (inappropriate for quantitative or qualitative use in risk assessment because it is of insufficient quality and lacks scientific defensibility).

Reference: WHO (updated 2005). Glyphosate and AMPA in drinking water. Background document for development of WHO *Guidelines for Drinking-water Quality*. Geneva, World Health Organization

http://www.who.int/water_sanitation_health/dwq/chemicals/glyphosate/en/

August 23, 2012

Note To/Note au: Toxicology File

From/De: 2032
Evaluation Officer, TRS-2, HED

Subject/Objet: **Sub. No(s):** 2004-0175
Product Name: Glyphosate products
Active Ingredient: Glyphosate acid and salt forms (GPS)

Action Requested : **Screen of Open Literature Studies**

Study Title: Safety Evaluation and Risk Assessment of the Herbicide Roundup and Its Active Ingredient, Glyphosate, for Humans

Summary: This is a review that analyses results from mostly regulatory studies and published research reports on Roundup formulation (referring to this entire family of formulations), and its ingredients: glyphosate, its major metabolite AMPA and predominant surfactant (POEA), focusing on risk assessment for humans.

Roundup has very low absorption in dermal studies. Direct ocular exposure to the concentrated formulation can result in transient irritation. There was no convincing evidence for direct DNA damage *in vitro* or *in vivo* with Round up and its components and it was concluded that there is no risk for the production of heritable/somatic mutations in humans. Roundup herbicide did not result in adverse effects on development, reproduction, or endocrine systems in humans or other mammals.

Glyphosate and AMPA have low oral absorption and glyphosate is eliminated essentially unmetabolized and does not bioaccumulate in animal tissues. Data from multiple lifetime feeding studies indicated that glyphosate is not carcinogenic. Glyphosate, AMPA and POEA were considered not teratogenic or developmentally toxic. Results from standard studies with glyphosate, AMPA and POEA also did not indicate endocrine modulation and there was no evidence of neurotoxicity.

No reliable evidence has been published that indicates a possible synergism with glyphosate and POEA. Roundup was concluded to not pose a health risk to humans if used under present and expected conditions of use.

AMPA details: Only 20% of AMPA was absorbed (biotransformed and 65% of it excreted in urine by 24hrs), and 74% of the administered dose was excreted in feces (by day 5). Trace residues were detected in the liver, kidney and skeletal muscle. Acute toxicity of AMPA was very low (LD50 if 8300 mg/kg bw), classified by the US EPA as category IV, the least toxic. Subchronic toxicity of AMPA has been investigated in rats and dogs, with treatment-related effects noted only at high doses. The NOAEL was 400 mg/kg bw/day (LOAEL of 1200 mg/kg bw/day based on urinary tract infection), for rats and NOAEL ≥263 mg/kg bw/day (no LOAEL) for dogs.

No lifetime studies were conducted with AMPA, only with glyphosate that contained 0.68% of AMPA. It was concluded that AMPA was not oncogenic at dosage levels up to 7.2 mg/kg bw/day, with a NOAEL for chronic effects of at least 2.8 mg/kg bw/day.

Similarly only glyphosate and 0.68% AMPA study was available for reproduction and developmental toxicity. The overall NOAEL for AMPA was considered to be ~ 4.3 mg/kg bw/day (based on systemic not reproductive toxicity) and 400 mg/kg bw/day for maternal and developmental toxicity.

POEA details: Acute toxicity of POEA was higher than that of Roundup formulation. The oral acute toxicity (LD 50~1200 mg/kg bw) would be likely categorized in the US EPA's second least-toxic category (III). POEA was reported to be severely irritating to the skin and corrosive to the eyes in rabbits, consistent with surface-active properties of surfactants (interacting and solubilizing lipid components characteristic of skin and mucous membranes).

In subchronic studies in rats, a NOAEL of ~36 mg/kg bw/day was established in rats. Main findings included reduced body weight gain, prominent/enlarged lymphoid aggregates in the colon (females) associated with direct irritation/inflammatory effect of POEA in a shorter rat study. In another study in rats, intestinal irritation, decreased food consumption and body weight gains, and some alterations in serum hematology/clinical chemistry parameters were observed. Gastrointestinal intolerance (emesis and diarrhea) was observed in dogs (NOAEL was not established), showing inability of these animals to tolerate surfactant ingestion on daily basis. Other findings included reduced body weights and slight reductions in serum calcium and protein.

In a developmental study, significant maternal toxicity was noted at the high dose, but minimal effects of decreased food consumption and clinical signs at lower doses, while there were no effects on fetuses ant the does testes. Developmental NOAEL was 300 mg/kg bw/day, and maternal was 15 mg/kg bw/day. POEA was not considered to be a teratogen or developmental toxin in rats.

No reproductive or chronic/oncogenicity studies with POEA were discussed.

Salivary Gland Changes:

Salivary gland changes were noted in rats with subchronic treatment with glyphosate. It was hypothesized that the alterations were caused by weak β-adrenergic activity of glyphosate. In a follow-up study, rats were given glyphosate or isoproterenol (a β-adrenergic agonist) treatment. Animals from both treatment groups were observed to have increased salivary gland weights. When isoproterenol was given with propranolol (β-blocker), the increase in salivary gland weight was not noted. If glyphosate were a β-agonist, it would stimulate β-receptors in other effector organs, but plausible cardiovascular effects, myocardial necrosis or enlargement of heart ventricles were not noted. Furthermore, glyphosate is not structurally related to known β-agonists. It was concluded that glyphosate does not act as β-agonist and has no significant β-adrenergic activity. However, it does not negate the observed effect that glyphosate has on salivary gland, just rejects the hypothesized mode of action.

Nonchemical mechanism of action for glyphosate was proposed. Glyphosate is a strong organic acid, and high levels may cause mild oral irritation leading to increased salivary gland size and flow. Because noted changes were (1) most pronounced in the parotid gland, responsible for secretion of serous fluid (possibly in response to such stimuli as acidic material), (2) absent in sublingual gland that releases mucous in response to other stimuli, (3) observed to an intermediate degree in submandibular gland that contains a mixture of mucous and serous secreting cells, salivary gland changes were likely a biological response to the acidic nature of treatment.

Salivary gland changes are arguably considered to be of doubtful toxicological significance, in the absence of other significant adverse effects at the doses used. The changes were not associated with any adverse clinical or pathological effect in chronic studies, and are not known to represent any pathologic condition and have no known as of yet relevance to humans.

Limitations:

- This is a review paper

Conclusion: This study does not meet the minimum criteria to be eligible for risk assessment of the active ingredient, as outlined in the draft "Guidance for considering and using open literature studies to support human health risk assessment" (US EPA, 2011). This study is categorized as 'invalid' since it is a review paper not a single study (inappropriate for quantitative or qualitative use in risk assessment because it is of insufficient quality and lacks scientific defensibility).

Flag: Salivary gland changes were discussed

Reference: Williams, Garry M., Kroes Robert, and Munro, Ian C. (1999). Safety Evaluation and Risk Assessment of the Herbicide Roundup and Its Active Ingredient, Glyphosate, for Humans. Regulatory toxicology and Pahrmacology. 31: 117-165.

Note To/Note au: Toxicology File

From/De: 2032
Evaluation Officer, TRS-2, HED

Subject/Objet: **Sub. No(s):** 2004-0175
Product Name: Glyphosate products
Active Ingredient: Glyphosate acid and salt forms (GPS)

Action Requested : **Screen of Open Literature Studies**

Study Title: Developmental and reproductive outcomes in humans and animals after glyphosate exposure: a critical analysis.

Summary: An analysis of available literature was conducted, including animal, mechanistic and epidemiologic studies, to assess possible developmental and reproductive effects of glyphosate. There was no consistent effect of exposure on reproductive health or the development of offspring. Toxicity was observed in some studies; however these studies used glyphosate formulation not the active ingredient itself. The exposure to the surfactant and/or other adjuvants, instead direct exposure to glyphosate was thought to contribute to the observed toxicity.

Biomonitoring data was examined to estimate occupational human exposure. The estimated concentration from normal application rates was found to be very low, approximately >500-fold less than the oral reference dose for glyphosate (2 mg/kg bw/day), as established by the US EPA (1993).

Authors concluded that there is no solid evidence linking glyphosate exposure to adverse developmental and reproductive effects at environmentally realistic exposure concentrations in the available literature.

Limitations:

- Review, not a single study.
- Most human studies involved formulations, and have poor characterization of the test substance.
- Some studies suffered from numerous inadequacies in design and reporting

Conclusion: This study does not meet the minimum criteria to be eligible for risk assessment of the active ingredient, as outlined in the draft "Guidance for considering and using open literature studies to support human health risk assessment" (US EPA, 2011). The major limitation as discussed above was the use of end products, not the active ingredients. This study is categorized as 'invalid' (inappropriate for quantitative or qualitative use in risk assessment because it is of insufficient quality and lacks scientific defensibility).

Reference: Amy Lavin Williams, Rebecca E. Watson & John M. DeSesso (2012): Developmental and Reproductive Outcomes in Humans and Animals After Glyphosate Exposure: A Critical Analysis, Journal of Toxicology and Environmental Health, Part B: Critical Reviews, 15:1, 39-96.

Appendix D: Literature studies obtained from searching PubMed for recent systematic review

Comments	Authors	PubDate (Year)	Full Citation
Not within scope/ecological and fate	Tatum VL, Borton DL, Streblow WR, Louch J, Shepard JP	2012	Tatum VL, Borton DL, Streblow WR, Louch J, Shepard JP.Acute toxicity of commonly used forestry herbicide mixtures to Ceriodaphnia dubia and Pimephales promelas. Environ Toxicol. 2012 Dec; 27(12):671-84.
Not within scope/ecological and fate	Hued AC, Oberhofer S, de los Ãngeles Bistoni M	2012	Hued AC, Oberhofer S, de los Ã ngeles Bistoni M.Exposure to a commercial glyphosate formulation (Roundup®) alters normal gill and liver histology and affects male sexual activity of Jenynsia multidentata (Anablepidae, Cyprinodontiformes). Arch Environ Contam Toxicol. 2012 Jan; 62(1):107-17.
Not within scope/ecological and fate	Coupe RH, Kalkhoff SJ, Capel PD, Gregoire C	2012	Coupe RH, Kalkhoff SJ, Capel PD, Gregoire C.Fate and transport of glyphosate and aminomethylphosphonic acid in surface waters of agricultural basins. Pest Manag Sci. 2012 Jan; 68(1):16-30.
Not within scope/ecological and fate	Sviridov AV, Shushkova TV, Zelenkova NF, Vinokurova NG, Morgunov IG, Ermakova IT, Leontievsky AA	2012	Sviridov AV, Shushkova TV, Zelenkova NF, Vinokurova NG, Morgunov IG, Ermakova IT, Leontievsky AA.Distribution of glyphosate and methylphosphonate catabolism systems in soil bacteria Ochrobactrum anthropi and Achromobacter sp. Appl Microbiol Biotechnol. 2012 Jan; 93(2):787-96.
Not within scope/ecological and fate	Hunsche M, Noga G	2012	Hunsche M, Noga G.Effects of relative humidity and substrate on the spatial association between glyphosate and ethoxylated seed oil adjuvants in the dried deposits of sessile droplets. Pest Manag Sci. 2012 Feb; 68(2):231-9.
Not within scope/ecological and fate	Negga R, Stuart JA, Machen ML, Salva J, Lizek AJ, Richardson SJ, Osborne AS, Mirallas O, McVey KA, Fitsanakis VA	2012	Negga R, Stuart JA, Machen ML, Salva J, Lizek AJ, Richardson SJ, Osborne AS, Mirallas O, McVey KA, Fitsanakis VA.Exposure to glyphosate- and/or Mn/Zn-ethylene-bis-dithiocarbamate-containing pesticides leads to degeneration of Î³-aminobutyric acid and dopamine neurons in Caenorhabditis elegans. Neurotox Res. 2012 Apr; 21(3):281-90.
Not within scope/ecological and fate	Glozier NE, Struger J, Cessna AJ, Gledhill M, Rondeau M, Ernst WR, Sekela MA, Cagampan SJ, Sverko E, Murphy C, Murray JL, Donald DB	2012	Glozier NE, Struger J, Cessna AJ, Gledhill M, Rondeau M, Ernst WR, Sekela MA, Cagampan SJ, Sverko E, Murphy C, Murray JL, Donald DB.Occurrence of glyphosate and acidic herbicides in select urban rivers and streams in Canada, 2007. Environ Sci Pollut Res Int. 2012 Mar; 19(3):821-34.

Comments	Authors	PubDate (Year)	Full Citation
Not within scope/ecological and fate	MenÃ©ndez-Helman RJ, Ferreyroa GV, dos Santos Afonso M, SalibiÃ¡n A	2012	MenÃ©ndez-Helman RJ, Ferreyroa GV, dos Santos Afonso M, SalibiÃ¡n A.Glyphosate as an acetylcholinesterase inhibitor in Cnesterodon decemmaculatus. Bull Environ Contam Toxicol. 2012 Jan; 88(1):6-9.
Not within scope/ecological and fate	Geret F, Burgeot T, Haure J, Gagnaire B, Renault T, Communal PY, Samain JF	2013	Geret F, Burgeot T, Haure J, Gagnaire B, Renault T, Communal PY, Samain JF.Effects of low-dose exposure to pesticide mixture on physiological responses of the Pacific oyster, Crassostrea gigas. Environ Toxicol. 2013 Dec; 28(12):689-99.
Not within scope/ecological and fate	Magga Z, Tzovolou DN, Theodoropoulou MA, Tsakiroglou CD	2012	Magga Z, Tzovolou DN, Theodoropoulou MA, Tsakiroglou CD.Combining experimental techniques with non-linear numerical models to assess the sorption of pesticides on soils. J Contam Hydrol. 2012 Mar 15; 129-130:62-9.
Not relevant	Tsai TW, Mou Y, Chan JC	2012	Tsai TW, Mou Y, Chan JC.Time displacement rotational echo double resonance: heteronuclear dipolar recoupling with suppression of homonuclear interaction under fast magic-angle spinning. J Magn Reson. 2012 Jan; 214(1):315-8.
Not within scope/ecological and fate	Botta F, Fauchon N, Blanchoud H, Chevreuil M, Guery B	2012	Botta F, Fauchon N, Blanchoud H, Chevreuil M, Guery B.Phyt'Eaux CitÃ©s: application and validation of a programme to reduce surface water contamination with urban pesticides. Chemosphere. 2012 Jan; 86(2):166-76.
Not within scope/ecological and fate	Demetrio PM, Bulus Rossini GD, Bonetto CA, Ronco AE	2012	Demetrio PM, Bulus Rossini GD, Bonetto CA, Ronco AE.Effects of pesticide formulations and active ingredients on the coelenterate Hydra attenuata (Pallas, 1766). Bull Environ Contam Toxicol. 2012 Jan; 88(1):15-9.
Not within scope/ecological and fate	SanchÃs J, Kantiani L, Llorca M, Rubio F, Ginebreda A, Fraile J, Garrido T, FarrÃ© M	2012	SanchÃs J, Kantiani L, Llorca M, Rubio F, Ginebreda A, Fraile J, Garrido T, FarrÃ© M.Determination of glyphosate in groundwater samples using an ultrasensitive immunoassay and confirmation by on-line solid-phase extraction followed by liquid chromatography coupled to tandem mass spectrometry. Anal Bioanal Chem. 2012 Mar; 402(7):2335-45.
Not within scope/ecological and fate	Akcha F, Spagnol C, Rouxel J	2012	Akcha F, Spagnol C, Rouxel J.Genotoxicity of diuron and glyphosate in oyster spermatozoa and embryos. Aquat Toxicol. 2012 Jan 15; 106-107:104-13.
Evaluated in search with PMRA (Appendix B)	Romano MA, Romano RM, Santos LD, Wisniewski P, Campos DA, de Souza PB, Viau P, Bernardi MM, Nunes MT, de Oliveira CA	2012	Romano MA, Romano RM, Santos LD, Wisniewski P, Campos DA, de Souza PB, Viau P, Bernardi MM, Nunes MT, de Oliveira CA.Glyphosate impairs male offspring reproductive development by disrupting gonadotropin expression. Arch Toxicol. 2012 Apr; 86(4):663-73.

Comments	Authors	PubDate (Year)	Full Citation
Not within scope/ecological and fate	Lashermes G, Barriuso E, Houot S	2012	Lashermes G, Barriuso E, Houot S.Dissipation pathways of organic pollutants during the composting of organic wastes. Chemosphere. 2012 Apr; 87(2):137-43.
Not within scope/ecological and fate	Rousseau AN, Lafrance P, Lavigne MP, Savary S, Konan B, QuilbÃ© R, Jiapizian P, Amrani M	2012	Rousseau AN, Lafrance P, Lavigne MP, Savary S, Konan B, QuilbÃ© R, Jiapizian P, Amrani M.A hydrological modeling framework for defining achievable performance standards for pesticides. J Environ Qual. 2012 Jan-Feb; 41(1):52-63.
Not within scope/ecological and fate	Wrinn KM, Evans SC, Rypstra AL	2012	Wrinn KM, Evans SC, Rypstra AL.Predator cues and an herbicide affect activity and emigration in an agrobiont wolf spider. Chemosphere. 2012 Apr; 87(4):390-6.
Not within scope/ecological and fate	Hanlon SM, Parris MJ	2012	Hanlon SM, Parris MJ.The impact of pesticides on the pathogen Batrachochytrium dendrobatidis independent of potential hosts. Arch Environ Contam Toxicol. 2012 Jul; 63(1):137-43.
Not within scope/ exposure and monitoring	McQueen H, Callan AC, Hinwood AL	2012	McQueen H, Callan AC, Hinwood AL.Estimating maternal and prenatal exposure to glyphosate in the community setting. Int J Hyg Environ Health. 2012 Nov; 215(6):570-6.
Not within scope/ecological and fate	Guilherme S, GaivÃ£o I, Santos MA, Pacheco M	2012	Guilherme S, GaivÃ£o I, Santos MA, Pacheco M.DNA damage in fish (Anguilla anguilla) exposed to a glyphosate-based herbicide -- elucidation of organ-specificity and the role of oxidative stress. Mutat Res. 2012 Mar 18; 743(1-2):1-9.
Not within scope/ecological and fate	HorÄ iÄ iak M, MasÃ¡r M, Bodor R, DanÄ L, Bel P	2012	HorÄ iÄ iak M, MasÃ¡r M, Bodor R, DanÄ L, Bel P.Trace analysis of glyphosate in water by capillary electrophoresis on a chip with high sample volume loadability. J Sep Sci. 2012 Mar; 35(5-6):674-80.
Poisoning or incident	Kamijo Y, Mekari M, Yoshimura K, Kan'o T, Soma K	2012	Kamijo Y, Mekari M, Yoshimura K, Kan'o T, Soma K.Glyphosate-surfactant herbicide products containing glyphosate potassium salt can cause fatal hyperkalemia if ingested in massive amounts. Clin Toxicol (Phila). 2012 Feb; 50(2):159.
In vitro/cytotox/no glyphosate measurements	Koller VJ, FÃ¼rhacker M, Nersesyan A, MiÅ¡Ãk M, Eisenbauer M, Knasmueller S	2012	Koller VJ, FÃ¼rhacker M, Nersesyan A, MiÅ¡Ãk M, Eisenbauer M, Knasmueller S.Cytotoxic and DNA-damaging properties of glyphosate and Roundup in human-derived buccal epithelial cells. Arch Toxicol. 2012 May; 86(5):805-13.

Comments	Authors	PubDate (Year)	Full Citation
Not within scope/ method generating	Liu ZY, Xie M, Ni F, Xu YH	2012	Liu ZY, Xie M, Ni F, Xu YH.Nanofiltration process of glyphosate simulated wastewater. Water Sci Technol. 2012; 65(5):816-22.
Not within scope/ methods generating	Wagner R, Wetzel SJ, Kern J, Kingston HM	2012	Wagner R, Wetzel SJ, Kern J, Kingston HM.Improved sample preparation of glyphosate and methylphosphonic acid by EPA method 6800A and time-of-flight mass spectrometry using novel solid-phase extraction. J Mass Spectrom. 2012 Feb; 47(2):147-54.
Microbiota/no glyphosate measurements	Clair E, Linn L, Travert C, Amiel C, SÃ©ralini GE, Panoff JM	2012	Clair E, Linn L, Travert C, Amiel C, SÃ©ralini GE, Panoff JM.Effects of Roundup(Â®) and glyphosate on three food microorganisms: Geotrichum candidum, Lactococcus lactis subsp. cremoris and Lactobacillus delbrueckii subsp. bulgaricus. Curr Microbiol. 2012 May; 64(5):486-91.
Not within scope/ecological and fate	Lessard CR, Frost PC	2012	Lessard CR, Frost PC.Phosphorus nutrition alters herbicide toxicity on Daphnia magna. Sci Total Environ. 2012 Apr 1; 421-422:124-8.
In vitro/not publicly available for free	Heu C, Berquand A, Elie-Caille C, Nicod L	2012	Heu C, Berquand A, Elie-Caille C, Nicod L.Glyphosate-induced stiffening of HaCaT keratinocytes, a Peak Force Tapping study on living cells. J Struct Biol. 2012 Apr; 178(1):1-7.
Not within scope/ecological and fate	Choi CJ, Berges JA, Young EB	2012	Choi CJ, Berges JA, Young EB.Rapid effects of diverse toxic water pollutants on chlorophyll a fluorescence: variable responses among freshwater microalgae. Water Res. 2012 May 15; 46(8):2615-26.
Not within scope/ecological and fate	Bonnineau C, Sague IG, Urrea G, Guasch H	2012	Bonnineau C, Sague IG, Urrea G, Guasch H.Light history modulates antioxidant and photosynthetic responses of biofilms to both natural (light) and chemical (herbicides) stressors. Ecotoxicology. 2012 May; 21(4):1208-24.
Not within scope/ecological and fate	Nourouzi MM, Chuah TG, Choong TS, Rabiei F	2012	Nourouzi MM, Chuah TG, Choong TS, Rabiei F.Modeling biodegradation and kinetics of glyphosate by artificial neural network. J Environ Sci Health B. 2012; 47(5):455-65.
Poisoning or incident	You Y, Jung WJ, Lee MJ	2012	You Y, Jung WJ, Lee MJ.Effect of intravenous fat emulsion therapy on glyphosate-surfactant-induced cardiovascular collapse. Am J Emerg Med. 2012 Nov; 30(9):2097.e1-2.

Comments	Authors	PubDate (Year)	Full Citation
Not within scope/ecological and fate	Wang G, Deng S, Li C, Liu Y, Chen L, Hu C	2012	Wang G, Deng S, Li C, Liu Y, Chen L, Hu C.Damage to DNA caused by UV-B radiation in the desert cyanobacterium Scytonema javanicum and the effects of exogenous chemicals on the process. Chemosphere. 2012 Jul; 88(4):413-7.
Not within scope/ecological and fate	Carrasco-Letelier L, Mendoza-Spina Y, Branchiccela MB	2012	Carrasco-Letelier L, Mendoza-Spina Y, Branchiccela MB.Acute contact toxicity test of insecticides (Cipermetrina 25, Lorsban 48E, Thionex 35) on honeybees in the southwestern zone of Uruguay. Chemosphere. 2012 Jul; 88(4):439-44.
Not relevant	Liu ZQ, Zhou M, Zhang XH, Xu JM, Xue YP, Zheng YG	2012	Liu ZQ, Zhou M, Zhang XH, Xu JM, Xue YP, Zheng YG.Biosynthesis of iminodiacetic acid from iminodiacetonitrile by immobilized recombinant Escherichia coli harboring nitrilase. J Mol Microbiol Biotechnol. 2012; 22(1):35-47.
In vitro/no glyphosate measurements/glyphosate had no effect	Forgacs AL, Ding Q, Jaremba RG, Huhtaniemi IT, Rahman NA, Zacharewski TR	2012	Forgacs AL, Ding Q, Jaremba RG, Huhtaniemi IT, Rahman NA, Zacharewski TR.BLTK1 murine Leydig cells: a novel steroidogenic model for evaluating the effects of reproductive and developmental toxicants. Toxicol Sci. 2012 Jun; 127(2):391-402.
Not within scope/ecological and fate	Chen L, Xie M, Bi Y, Wang G, Deng S, Liu Y	2012	Chen L, Xie M, Bi Y, Wang G, Deng S, Liu Y.The combined effects of UV-B radiation and herbicides on photosynthesis, antioxidant enzymes and DNA damage in two bloom-forming cyanobacteria. Ecotoxicol Environ Saf. 2012 Jun; 80:224-30.
Not within scope/ecological and fate	Uchida M, Takumi S, Tachikawa K, Yamauchi R, Goto Y, Matsusaki H, Nakamura H, Kagami Y, Kusano T, Arizono K	2012	Uchida M, Takumi S, Tachikawa K, Yamauchi R, Goto Y, Matsusaki H, Nakamura H, Kagami Y, Kusano T, Arizono K.Toxicity evaluation of glyphosate agrochemical components using Japanese medaka (Oryzias latipes) and DNA microarray gene expression analysis. J Toxicol Sci. 2012; 37(2):245-54.
Not within scope/ecological and fate	Chen JQ, Hu ZJ, Wang NX	2012	Chen JQ, Hu ZJ, Wang NX.Photocatalytic mineralization of glyphosate in a small-scale plug flow simulation reactor by UV/TiO2. J Environ Sci Health B. 2012; 47(6):579-88.
In vitro/no glyphosate measurements	Gui YX, Fan XN, Wang HM, Wang G, Chen SD	2012	Gui YX, Fan XN, Wang HM, Wang G, Chen SD.Glyphosate induced cell death through apoptotic and autophagic mechanisms. Neurotoxicol Teratol. 2012 May-Jun; 34(3):344-9.
Not within scope/ecological and fate	Mensah PK, Palmer CG, Muller WJ	2012	Mensah PK, Palmer CG, Muller WJ.Lipid peroxidation in the freshwater shrimp Caridina nilotica as a biomarker of Roundup(Â®) herbicide pollution of freshwater systems in South Africa. Water Sci Technol. 2012; 65(9):1660-6.

Comments	Authors	PubDate (Year)	Full Citation
In vitro/no glyphosate measurements	Heu C, Elie-Caille C, Mougey V, Launay S, Nicod L	2012	Heu C, Elie-Caille C, Mougey V, Launay S, Nicod L.A step further toward glyphosate-induced epidermal cell death: involvement of mitochondrial and oxidative mechanisms. Environ Toxicol Pharmacol. 2012 Sep; 34(2):144-53.
Not within scope/ecological and fate	Guilherme S, Santos MA, Barroso C, GaivÃ£o I, Pacheco M	2012	Guilherme S, Santos MA, Barroso C, GaivÃ£o I, Pacheco M.Differential genotoxicity of Roundup(Â®) formulation and its constituents in blood cells of fish (Anguilla anguilla): considerations on chemical interactions and DNA damaging mechanisms. Ecotoxicology. 2012 Jul; 21(5):1381-90.
Not within scope/ecological and fate	Vera MS, Di Fiori E, Lagomarsino L, Sinistro R, Escaray R, Iummato MM, JuÃ¡rez A, RÃos de Molina Mdel C, Tell G, Pizarro H	2012	Vera MS, Di Fiori E, Lagomarsino L, Sinistro R, Escaray R, Iummato MM, JuÃ¡rez A, RÃos de Molina Mdel C, Tell G, Pizarro H.Direct and indirect effects of the glyphosate formulation Glifosato AtanorÂ® on freshwater microbial communities. Ecotoxicology. 2012 Oct; 21(7):1805-16.
In vitro/formulation/no glyphosate measurements	Martini CN, Gabrielli M, Vila Mdel C	2012	Martini CN, Gabrielli M, Vila Mdel C.A commercial formulation of glyphosate inhibits proliferation and differentiation to adipocytes and induces apoptosis in 3T3-L1 fibroblasts. Toxicol In Vitro. 2012 Sep; 26(6):1007-13.
Not within scope/ecological and fate	Degenhardt D, Humphries D, Cessna AJ, Messing P, Badiou PH, Raina R, Farenhorst A, Pennock DJ	2012	Degenhardt D, Humphries D, Cessna AJ, Messing P, Badiou PH, Raina R, Farenhorst A, Pennock DJ.Dissipation of glyphosate and aminomethylphosphonic acid in water and sediment of two Canadian prairie wetlands. J Environ Sci Health B. 2012; 47(7):631-9.
Not within scope/ecological and fate	Sura S, Waiser M, Tumber V, Lawrence JR, Cessna AJ, Glozier N	2012	Sura S, Waiser M, Tumber V, Lawrence JR, Cessna AJ, Glozier N.Effects of glyphosate and two herbicide mixtures on microbial communities in prairie wetland ecosystems: a mesocosm approach. J Environ Qual. 2012 May-Jun; 41(3):732-43.
Editorial	BellÃ© R, Marc J, Morales J, Cormier P, Mulner-Lorillon O	2012	BellÃ© R, Marc J, Morales J, Cormier P, Mulner-Lorillon O.Letter to the editor: toxicity of Roundup and glyphosate. J Toxicol Environ Health B Crit Rev. 2012; 15(4):233-5; author reply 236-7.
Editorial	Reding MA	2012	Reding MA.Letter to the editor regarding Determination of glyphosate in groundwater samples using an ultrasensitive immunoassay and confirmation by on-line solid phase extraction followed by liquid chromatography coupled to tandem mass spectrometry'. Anal Bioanal Chem. 2012 Aug; 404(2):613-4; author reply 615-6.

Comments	Authors	PubDate (Year)	Full Citation
Not within scope/ methods generating	Mazzei P, Piccolo A	2012	Mazzei P, Piccolo A.Quantitative evaluation of noncovalent interactions between glyphosate and dissolved humic substances by NMR spectroscopy. Environ Sci Technol. 2012 Jun 5; 46(11):5939-46.
Not within scope/ecological and fate	Di Fiori E, Pizarro H, dos Santos Afonso M, Cataldo D	2012	Di Fiori E, Pizarro H, dos Santos Afonso M, Cataldo D.Impact of the invasive mussel Limnoperna fortunei on glyphosate concentration in water. Ecotoxicol Environ Saf. 2012 Jul; 81:106-13.
Not within scope/ecological and fate	Shushkova TV, Ermakova IT, Sviridov AV, Leont'evskiÄ AA	2012	Shushkova TV, Ermakova IT, Sviridov AV, Leont'evskiÄ AA.[Biodegradation of glyphosate by soil bacteria: optimization of culture and method for active biomass preservation]. Mikrobiologiia. 2012 Jan-Feb; 81(1):48-55.
Not within scope/ecological and fate	Imfeld G, Lefrancq M, Maillard E, Payraudeau S	2013	Imfeld G, Lefrancq M, Maillard E, Payraudeau S.Transport and attenuation of dissolved glyphosate and AMPA in a stormwater wetland. Chemosphere. 2013 Jan; 90(4):1333-9.
In vitro/no glyphosate measurements/glyphosate used as neg. control	Culbreth ME, Harrill JA, Freudenrich TM, Mundy WR, Shafer TJ	2012	Culbreth ME, Harrill JA, Freudenrich TM, Mundy WR, Shafer TJ.Comparison of chemical-induced changes in proliferation and apoptosis in human and mouse neuroprogenitor cells. Neurotoxicology. 2012 Dec; 33(6):1499-510.
Not within scope/ecological and fate	Muangphra P, Kwankua W, Gooneratne R	2014	Muangphra P, Kwankua W, Gooneratne R.Genotoxic effects of glyphosate or paraquat on earthworm coelomocytes. Environ Toxicol. 2014 Jun; 29(6):612-20.
Not within scope/ecological and fate	Harris WR, Sammons RD, Grabiak RC, Mehrsheikh A, Bleeke MS	2012	Harris WR, Sammons RD, Grabiak RC, Mehrsheikh A, Bleeke MS.Computer simulation of the interactions of glyphosate with metal ions in phloem. J Agric Food Chem. 2012 Jun 20; 60(24):6077-87.
Not within scope/ methods generating	McConnell ER, McClain MA, Ross J, Lefew WR, Shafer TJ	2012	McConnell ER, McClain MA, Ross J, Lefew WR, Shafer TJ.Evaluation of multi-well microelectrode arrays for neurotoxicity screening using a chemical training set. Neurotoxicology. 2012 Oct; 33(5):1048-57.
Not within scope/ecological and fate	SaitÃºa H, Giannini F, Padilla AP	2012	SaitÃºa H, Giannini F, Padilla AP.Drinking water obtaining by nanofiltration from waters contaminated with glyphosate formulations: process evaluation by means of toxicity tests and studies on operating parameters. J Hazard Mater. 2012 Aug 15; 227-228:204-10.

Comments	Authors	PubDate (Year)	Full Citation
Not relevant	Zhou J, Li J, An R, Yuan H, Yu F	2012	Zhou J, Li J, An R, Yuan H, Yu F.Study on a new synthesis approach of glyphosate. J Agric Food Chem. 2012 Jun 27; 60(25):6279-85.
Poisoning or incident	Hour BT, Belen C, Zar T, Lien YH	2012	Hour BT, Belen C, Zar T, Lien YH.Herbicide roundup intoxication: successful treatment with continuous renal replacement therapy. Am J Med. 2012 Aug; 125(8):e1-2.
Epidemiology	Mink PJ, Mandel JS, Sceurman BK, Lundin JI	2012	Mink PJ, Mandel JS, Sceurman BK, Lundin JI.Epidemiologic studies of glyphosate and cancer: a review. Regul Toxicol Pharmacol. 2012 Aug; 63(3):440-52.
Not within scope/ecological and fate	Santos MJ, Ferreira MF, Cachada A, Duarte AC, Sousa JP	2012	Santos MJ, Ferreira MF, Cachada A, Duarte AC, Sousa JP.Pesticide application to agricultural fields: effects on the reproduction and avoidance behaviour of Folsomia candida and Eisenia andrei. Ecotoxicology. 2012 Nov; 21(8):2113-22.
Editorial/commentary	DeSesso JM, Williams AL	2012	DeSesso JM, Williams AL.Comment on Glyphosate impairs male offspring reproductive development by disrupting gonadotropin expression' by Romano et al. 2012. Arch Toxicol. 2012 Nov; 86(11):1791-3; author reply 1795-7.
Not within scope/ecological and fate	Shiogiri NS, Paulino MG, Carraschi SP, Baraldi FG, da Cruz C, Fernandes MN	2012	Shiogiri NS, Paulino MG, Carraschi SP, Baraldi FG, da Cruz C, Fernandes MN.Acute exposure of a glyphosate-based herbicide affects the gills and liver of the Neotropical fish, Piaractus mesopotamicus. Environ Toxicol Pharmacol. 2012 Sep; 34(2):388-96.
Not relevant	Pouessel J, Le Bris N, Bencini A, Giorgi C, Valtancoli B, Tripier R	2012	Pouessel J, Le Bris N, Bencini A, Giorgi C, Valtancoli B, Tripier R.Glyphosate and ATP binding by mononuclear Zn(II) complexes with non-symmetric ditopic polyamine ligands. Dalton Trans. 2012 Sep 21; 41(35):10521-32.
In vitro/cytotox/no glyphosate measurements	Song HY, Kim YH, Seok SJ, Gil HW, Hong SY	2012	Song HY, Kim YH, Seok SJ, Gil HW, Hong SY.In vitro cytotoxic effect of glyphosate mixture containing surfactants. J Korean Med Sci. 2012 Jul; 27(7):711-5.
Not within scope/ exposure and monitoring	Hu Z, Ye M, Pan G, Zhang T, Zhong N	2012	Hu Z, Ye M, Pan G, Zhang T, Zhong N.[Simultaneous determination of phosphorous by-products and inorganic anions in glyphosate mother liquor by ion chromatography with suppressed-conductivity detection]. Se Pu. 2012 Apr; 30(4):391-4.
Not within scope/ exposure and monitoring	Wang Y, Wu B, Lian H, Shi C	2012	Wang Y, Wu B, Lian H, Shi C.[Determination of glyphosate in heart blood of corpse by ion chromatography]. Se Pu. 2012 Apr; 30(4):419-22.

Comments	Authors	PubDate (Year)	Full Citation
Not within scope/ methods generating	Rathod R, Kang Z, Hartson SD, Kumauchi M, Xie A, Hoff WD	2012	Rathod R, Kang Z, Hartson SD, Kumauchi M, Xie A, Hoff WD.Side-chain specific isotopic labeling of proteins for infrared structural biology: the case of ring-D4-tyrosine isotope labeling of photoactive yellow protein. Protein Expr Purif. 2012 Sep; 85(1):125-32.
Not within scope/ecological and fate	de Castilhos Ghisi N, Cestari MM	2013	de Castilhos Ghisi N, Cestari MM.Genotoxic effects of the herbicide Roundup(Â®) in the fish Corydoras paleatus (Jenyns 1842) after short-term, environmentally low concentration exposure. Environ Monit Assess. 2013 Apr; 185(4):3201-7.
Poisoning or incident	Sribanditmongkol P, Jutavijittum P, Pongraveevongsa P, Wunnapuk K, Durongkadech P	2012	Sribanditmongkol P, Jutavijittum P, Pongraveevongsa P, Wunnapuk K, Durongkadech P.Pathological and toxicological findings in glyphosate-surfactant herbicide fatality: a case report. Am J Forensic Med Pathol. 2012 Sep; 33(3):234-7.
Not within scope/ecological and fate	Hanana H, Simon G, Kervarec N, Mohammadou BA, CÃ©rantola S	2012	Hanana H, Simon G, Kervarec N, Mohammadou BA, CÃ©rantola S.HRMAS NMR as a tool to study metabolic responses in heart clam Ruditapes decussatus exposed to RoundupÂ®. Talanta. 2012 Aug 15; 97:425-31.
Not within scope/ecological and fate	Sura S, Waiser M, Tumber V, Farenhorst A	2012	Sura S, Waiser M, Tumber V, Farenhorst A.Effects of herbicide mixture on microbial communities in prairie wetland ecosystems: a whole wetland approach. Sci Total Environ. 2012 Oct 1; 435-436:34-43.
Not within scope/ methods generating	Liu Z, Liu S, Yin P, He Y	2012	Liu Z, Liu S, Yin P, He Y.Fluorescence enhancement of CdTe/CdS quantum dots by coupling of glyphosate and its application for sensitive detection of copper ion. Anal Chim Acta. 2012 Oct 1; 745:78-84.
Not within scope/ecological and fate	Zhou CF, Wang YJ, Yu YC, Sun RJ, Zhu XD, Zhang HL, Zhou DM	2012	Zhou CF, Wang YJ, Yu YC, Sun RJ, Zhu XD, Zhang HL, Zhou DM.Does glyphosate impact on Cu uptake by, and toxicity to, the earthworm Eisenia fetida? Ecotoxicology. 2012 Nov; 21(8):2297-305.
Not within scope/ecological and fate	Fan J, Yang G, Zhao H, Shi G, Geng Y, Hou T, Tao K	2012	Fan J, Yang G, Zhao H, Shi G, Geng Y, Hou T, Tao K.Isolation, identification and characterization of a glyphosate-degrading bacterium, Bacillus cereus CB4, from soil. J Gen Appl Microbiol. 2012; 58(4):263-71.
In vitro/pesticide mixture	Astiz M, de Alaniz MJ, Marra CA	2012	Astiz M, de Alaniz MJ, Marra CA.The oxidative damage and inflammation caused by pesticides are reverted by lipoic acid in rat brain. Neurochem Int. 2012 Dec; 61(7):1231-41.

Comments	Authors	PubDate (Year)	Full Citation
In vitro/cytotox/no glyphosate measurements	Mesnage R, Bernay B, SÃ©ralini GE	2013	Mesnage R, Bernay B, SÃ©ralini GE.Ethoxylated adjuvants of glyphosate-based herbicides are active principles of human cell toxicity. Toxicology. 2013 Nov 16; 313(2-3):122-8.
Enzyme activity/no gylphosate measurements/no histopath changes observed in liver, kidney, and small intestine	Larsen K, Najle R, Lifschitz A, Virkel G	2012	Larsen K, Najle R, Lifschitz A, Virkel G.Effects of sub-lethal exposure of rats to the herbicide glyphosate in drinking water: glutathione transferase enzyme activities, levels of reduced glutathione and lipid peroxidation in liver, kidneys and small intestine. Environ Toxicol Pharmacol. 2012 Nov; 34(3):811-8.
Not within scope/ecological and fate	Kylin H	2013	Kylin H.Time-integrated sampling of glyphosate in natural waters. Chemosphere. 2013 Feb; 90(6):1821-8.
Not within scope/ methods generating	Costa JC, Ando RA, Sant'Ana AC, Corio P	2012	Costa JC, Ando RA, Sant'Ana AC, Corio P.Surface-enhanced Raman spectroscopy studies of organophosphorous model molecules and pesticides. Phys Chem Chem Phys. 2012 Dec 5; 14(45):15645-51.
Not within scope/ecological and fate	Anbalagan C, Lafayette I, Antoniou-Kourounioti M, Gutierrez C, Martin JR, Chowdhuri DK, De Pomerai DI	2013	Anbalagan C, Lafayette I, Antoniou-Kourounioti M, Gutierrez C, Martin JR, Chowdhuri DK, De Pomerai DI.Use of transgenic GFP reporter strains of the nematode Caenorhabditis elegans to investigate the patterns of stress responses induced by pesticides and by organic extracts from agricultural soils. Ecotoxicology. 2013 Jan; 22(1):72-85.
Not within scope/ecological and fate	Mbanaso FU, Coupe SJ, Charlesworth SM, Nnadi EO	2013	Mbanaso FU, Coupe SJ, Charlesworth SM, Nnadi EO.Laboratory-based experiments to investigate the impact of glyphosate-containing herbicide on pollution attenuation and biodegradation in a model pervious paving system. Chemosphere. 2013 Jan; 90(2):737-46.
In vitro/no glyphosate measurements	Kim YH, Hong JR, Gil HW, Song HY, Hong SY	2013	Kim YH, Hong JR, Gil HW, Song HY, Hong SY.Mixtures of glyphosate and surfactant TN20 accelerate cell death via mitochondrial damage-induced apoptosis and necrosis. Toxicol In Vitro. 2013 Feb; 27(1):191-7.

Comments	Authors	PubDate (Year)	Full Citation
Not within scope/ methods generating	Padilla-SÃ¡nchez JA, Plaza-BolaÃ±os P, Romero-GonzÃ¡lez R, Grande-MartÃnez A, Thurman EM, Garrido-Frenich A	2012	Padilla-SÃ¡nchez JA, Plaza-BolaÃ±os P, Romero-GonzÃ¡lez R, Grande-MartÃnez A, Thurman EM, Garrido-Frenich A.Innovative determination of polar organophosphonate pesticides based on high-resolution Orbitrap mass spectrometry. J Mass Spectrom. 2012 Nov; 47(11):1458-65.
Not within scope/ecological and fate	Petersen J, Grant R, Larsen SE, Blicher-Mathiesen G	2012	Petersen J, Grant R, Larsen SE, Blicher-Mathiesen G.Sampling of herbicides in streams during flood events. J Environ Monit. 2012 Dec; 14(12):3284-94.
Editorial	Defarge N, Mesnage R, Gress S, SÃ©ralini GE	2012	Defarge N, Mesnage R, Gress S, SÃ©ralini GE.Letter to the editor: developmental and reproductive outcomes of roundup and glyphosate in humans and animals. J Toxicol Environ Health B Crit Rev. 2012; 15(7):433-7; author reply 438-40.
Microbiota/formulation/ no glyphosate measurements	Shehata AA, SchrÃ¶dl W, Aldin AA, Hafez HM, KrÃ¼ger M	2013	Shehata AA, SchrÃ¶dl W, Aldin AA, Hafez HM, KrÃ¼ger M.The effect of glyphosate on potential pathogens and beneficial members of poultry microbiota in vitro. Curr Microbiol. 2013 Apr; 66(4):350-8.
Not within scope/ecological and fate	Cuhra M, Traavik T, BÃ¸hn T	2013	Cuhra M, Traavik T, BÃ¸hn T.Clone- and age-dependent toxicity of a glyphosate commercial formulation and its active ingredient in Daphnia magna. Ecotoxicology. 2013 Mar; 22(2):251-62.
Not within scope/ecological and fate	Cui H, Li Q, Qian Y, Zhang Q, Zhai J	2012	Cui H, Li Q, Qian Y, Zhang Q, Zhai J.Preparation and adsorption performance of MnO2/PAC composite towards aqueous glyphosate. Environ Technol. 2012 Sep; 33(16-18):2049-56.
Not within scope/ecological and fate	Latorre MA, LÃ³pez GonzÃ¡lez EC, Larriera A, Poletta GL, Siroski PA	2013	Latorre MA, LÃ³pez GonzÃ¡lez EC, Larriera A, Poletta GL, Siroski PA.Effects of in vivo exposure to RoundupÂ® on immune system of Caiman latirostris. J Immunotoxicol. 2013 Oct-Dec; 10(4):349-54.
Not within scope/ecological and fate	Bois P, Huguenot D, JÃ©zÃ©quel K, Lollier M, Cornu JY, Lebeau T	2013	Bois P, Huguenot D, JÃ©zÃ©quel K, Lollier M, Cornu JY, Lebeau T.Herbicide mitigation in microcosms simulating stormwater basins subject to polluted water inputs. Water Res. 2013 Mar 1; 47(3):1123-35.
Effect on E. coli/not publicly available for free	Lu W, Li L, Chen M, Zhou Z, Zhang W, Ping S, Yan Y, Wang J, Lin M	2013	Lu W, Li L, Chen M, Zhou Z, Zhang W, Ping S, Yan Y, Wang J, Lin M.Genome-wide transcriptional responses of Escherichia coli to glyphosate, a potent inhibitor of the shikimate pathway enzyme 5-enolpyruvylshikimate-3-phosphate synthase. Mol Biosyst. 2013 Mar; 9(3):522-30.

Comments	Authors	PubDate (Year)	Full Citation
Not within scope/ methods generating	Bataller R, Campos I, Laguarda-Miro N, AlcaÃ±iz M, Soto J, MartÃnez-MÃ¡Ã±ez R, Gil L, GarcÃa-Breijo E, IbÃ¡Ã±ez-Civera J	2012	Bataller R, Campos I, Laguarda-Miro N, AlcaÃ±iz M, Soto J, MartÃnez-MÃ¡Ã±ez R, Gil L, GarcÃa-Breijo E, IbÃ¡Ã±ez-Civera J.Glyphosate detection by means of a voltammetric electronic tongue and discrimination of potential interferents. Sensors (Basel). 2012 Dec 18; 12(12):17553-68.
Not within scope/ecological and fate	Le Mer C, Roy RL, Pellerin J, Couillard CM, Maltais D	2013	Le Mer C, Roy RL, Pellerin J, Couillard CM, Maltais D.Effects of chronic exposures to the herbicides atrazine and glyphosate to larvae of the threespine stickleback (Gasterosteus aculeatus). Ecotoxicol Environ Saf. 2013 Mar; 89:174-81.
Not within scope/ecological and fate	Vera-Candioti J, Soloneski S, Larramendy ML	2013	Vera-Candioti J, Soloneski S, Larramendy ML.Evaluation of the genotoxic and cytotoxic effects of glyphosate-based herbicides in the ten spotted live-bearer fish Cnesterodon decemmaculatus (Jenyns, 1842). Ecotoxicol Environ Saf. 2013 Mar; 89:166-73.
Poisoning or incident	Deo SP, Shetty P	2012	Deo SP, Shetty P.Accidental chemical burns of oral mucosa by herbicide. JNMA J Nepal Med Assoc. 2012 Jan-Mar; 52(185):40-2.
Not within scope/ecological and fate	Ghafoor A, Jarvis NJ, StenstrÃ¶m J	2013	Ghafoor A, Jarvis NJ, StenstrÃ¶m J.Modelling pesticide sorption in the surface and subsurface soils of an agricultural catchment. Pest Manag Sci. 2013 Aug; 69(8):919-29.
Review	Kimmel GL, Kimmel CA, Williams AL, DeSesso JM	2013	Kimmel GL, Kimmel CA, Williams AL, DeSesso JM.Evaluation of developmental toxicity studies of glyphosate with attention to cardiovascular development. Crit Rev Toxicol. 2013 Feb; 43(2):79-95.
Poisoning or incident	KneÅ¾eviÄ‡ V, BoÅ¾iÄ‡ D, BudoÅ¡an I, ÄŒeliÄ‡ D, MiloÅ¡eviÄ‡ A, MitiÄ‡ I	2012	KneÅ¾eviÄ‡ V, BoÅ¾iÄ‡ D, BudoÅ¡an I, ÄŒeliÄ‡ D, MiloÅ¡eviÄ‡ A, MitiÄ‡ I.[Early continuous dialysis in acute glyphosate-surfactant poisoning]. Srp Arh Celok Lek. 2012 Sep-Oct; 140(9-10):648-52.
Not within scope/ exposure and monitoring	Zouaoui K, Dulaurent S, Gaulier JM, Moesch C, LachÃ¢tre G	2013	Zouaoui K, Dulaurent S, Gaulier JM, Moesch C, LachÃ¢tre G.Determination of glyphosate and AMPA in blood and urine from humans: about 13 cases of acute intoxication. Forensic Sci Int. 2013 Mar 10; 226(1-3):e20-5.
Not within scope/ methods generating	Kujawinski DM, Wolbert JB, Zhang L, Jochmann MA, Widory D, Baran N, Schmidt TC	2013	Kujawinski DM, Wolbert JB, Zhang L, Jochmann MA, Widory D, Baran N, Schmidt TC.Carbon isotope ratio measurements of glyphosate and AMPA by liquid chromatography coupled to isotope ratio mass spectrometry. Anal Bioanal Chem. 2013 Mar; 405(9):2869-78.

Comments	Authors	PubDate (Year)	Full Citation
Not within scope/ methods generating	Botero-Coy AM, IbÃ¡Ã±ez M, Sancho JV, HernÃ¡ndez F	2013	Botero-Coy AM, IbÃ¡Ã±ez M, Sancho JV, HernÃ¡ndez F.Improvements in the analytical methodology for the residue determination of the herbicide glyphosate in soils by liquid chromatography coupled to mass spectrometry. J Chromatogr A. 2013 May 31; 1292:132-41.
Not within scope/ecological and fate	Piola L, Fuchs J, Oneto ML, Basack S, Kesten E, CasabÃ© N	2013	Piola L, Fuchs J, Oneto ML, Basack S, Kesten E, CasabÃ© N.Comparative toxicity of two glyphosate-based formulations to Eisenia andrei under laboratory conditions. Chemosphere. 2013 Apr; 91(4):545-51.
Not within scope/ecological and fate	Hadi F, Mousavi A, Noghabi KA, Tabar HG, Salmanian AH	2013	Hadi F, Mousavi A, Noghabi KA, Tabar HG, Salmanian AH.New bacterial strain of the genus Ochrobactrum with glyphosate-degrading activity. J Environ Sci Health B. 2013; 48(3):208-13.
Not within scope/ecological and fate	Qiu H, Geng J, Ren H, Xia X, Wang X, Yu Y	2013	Qiu H, Geng J, Ren H, Xia X, Wang X, Yu Y.Physiological and biochemical responses of Microcystis aeruginosa to glyphosate and its RoundupÂ® formulation. J Hazard Mater. 2013 Mar 15; 248-249:172-6.
Poisoning or incident	Mariager TP, Madsen PV, EbbehÃ¸j NE, Schmidt B, Juhl A	2013	Mariager TP, Madsen PV, EbbehÃ¸j NE, Schmidt B, Juhl A.Severe adverse effects related to dermal exposure to a glyphosate-surfactant herbicide. Clin Toxicol (Phila). 2013 Feb; 51(2):111-3.
Not relevant	Pouessel J, Abada S, Le Bris N, Elhabiri M, CharbonniÃ¨re LJ, Tripier R	2013	Pouessel J, Abada S, Le Bris N, Elhabiri M, CharbonniÃ¨re LJ, Tripier R.A new bis-tetraamine ligand with a chromophoric 4-(9-anthracenyl)-2,6-dimethylpyridinyl linker for glyphosate and ATP sensing. Dalton Trans. 2013 Apr 14; 42(14):4859-72.
Not within scope/ecological and fate	Waiman CV, Avena MJ, Regazzoni AE, Zanini GP	2013	Waiman CV, Avena MJ, Regazzoni AE, Zanini GP.A real time in situ ATR-FTIR spectroscopic study of glyphosate desorption from goethite as induced by phosphate adsorption: effect of surface coverage. J Colloid Interface Sci. 2013 Mar 15; 394:485-9.
In vitro/no glyphosate measurements	KÄ±lÄ±nÃ§ N, Ä°ÅŸgÃ¶r MM, ÅžengÃ¼l B, Beydemir Åž	2015	KÄ±lÄ±nÃ§ N, Ä°ÅŸgÃ¶r MM, ÅžengÃ¼l B, Beydemir Åž.Influence of pesticide exposure on carbonic anhydrase II from sheep stomach. Toxicol Ind Health. 2015 Sep; 31(9):823-30.
Not within scope/ methods generating	See HH, Stratz S, Hauser PC	2013	See HH, Stratz S, Hauser PC.Electro-driven extraction across a polymer inclusion membrane in a flow-through cell. J Chromatogr A. 2013 Jul 26; 1300:79-84.

Comments	Authors	PubDate (Year)	Full Citation
Microbiota/formulation/ no glyphosate measurements	KrÃ¼ger M, Shehata AA, SchrÃ¶dl W, Rodloff A	2013	KrÃ¼ger M, Shehata AA, SchrÃ¶dl W, Rodloff A.Glyphosate suppresses the antagonistic effect of Enterococcus spp. on Clostridium botulinum. Anaerobe. 2013 Apr; 20:74-8.
Not within scope/ methods generating	Song J, Li XM, Figoli A, Huang H, Pan C, He T, Jiang B	2013	Song J, Li XM, Figoli A, Huang H, Pan C, He T, Jiang B.Composite hollow fiber nanofiltration membranes for recovery of glyphosate from saline wastewater. Water Res. 2013 Apr 15; 47(6):2065-74.
Not within scope/ecological and fate	Sandrini JZ, Rola RC, Lopes FM, Buffon HF, Freitas MM, Martins Cde M, da Rosa CE	2013	Sandrini JZ, Rola RC, Lopes FM, Buffon HF, Freitas MM, Martins Cde M, da Rosa CE.Effects of glyphosate on cholinesterase activity of the mussel Perna perna and the fish Danio rerio and Jenynsia multidentata: in vitro studies. Aquat Toxicol. 2013 Apr 15; 130-131:171-3.
Model application	Ramachandran V, Singh R, Yang X, Tunduguru R, Mohapatra S, Khandelwal S, Patel S, Datta S	2013	Ramachandran V, Singh R, Yang X, Tunduguru R, Mohapatra S, Khandelwal S, Patel S, Datta S.Genetic and chemical knockdown: a complementary strategy for evaluating an anti-infective target. Adv Appl Bioinform Chem. 2013; 6:1-13.
Pesticide mixture/no glyphosate measurements	Astiz M, Hurtado de Catalfo GE, GarcÃa MN, Galletti SM, Errecalde AL, de Alaniz MJ, Marra CA	2013	Astiz M, Hurtado de Catalfo GE, GarcÃa MN, Galletti SM, Errecalde AL, de Alaniz MJ, Marra CA.Pesticide-induced decrease in rat testicular steroidogenesis is differentially prevented by lipoate and tocopherol. Ecotoxicol Environ Saf. 2013 May; 91:129-38.
Not within scope/ecological and fate	Lagator M, Vogwill T, Colegrave N, Neve P	2013	Lagator M, Vogwill T, Colegrave N, Neve P.Herbicide cycling has diverse effects on evolution of resistance in Chlamydomonas reinhardtii. Evol Appl. 2013 Feb; 6(2):197-206.
Not within scope/ecological and fate	Janssens L, Stoks R	2013	Janssens L, Stoks R.Synergistic effects between pesticide stress and predator cues: conflicting results from life history and physiology in the damselfly Enallagma cyathigerum. Aquat Toxicol. 2013 May 15; 132-133:92-9.
Review	Kier LD, Kirkland DJ	2013	Kier LD, Kirkland DJ.Review of genotoxicity studies of glyphosate and glyphosate-based formulations. Crit Rev Toxicol. 2013 Apr; 43(4):283-315.
Not within scope/ecological and fate	Vialle C, Sablayrolles C, Silvestre J, Monier L, Jacob S, Huau MC, Montrejaud-Vignoles M	2013	Vialle C, Sablayrolles C, Silvestre J, Monier L, Jacob S, Huau MC, Montrejaud-Vignoles M.Pesticides in roof runoff: study of a rural site and a suburban site. J Environ Manage. 2013 May 15; 120:48-54.

Comments	Authors	PubDate (Year)	Full Citation
In vitro/no glyphosate measurements	Chorfa A, BÃ©temps D, Morignat E, Lazizzera C, Hogeveen K, Andrieu T, Baron T	2013	Chorfa A, BÃ©temps D, Morignat E, Lazizzera C, Hogeveen K, Andrieu T, Baron T.Specific pesticide-dependent increases in Î±-synuclein levels in human neuroblastoma (SH-SY5Y) and melanoma (SK-MEL-2) cell lines. Toxicol Sci. 2013 Jun; 133(2):289-97.
Not within scope/ecological and fate	Haudin CS, Zhang Y, DumÃ©ny V, Lashermes G, Bergheaud V, Barriuso E, Houot S	2013	Haudin CS, Zhang Y, DumÃ©ny V, Lashermes G, Bergheaud V, Barriuso E, Houot S.Fate of (14)C-organic pollutant residues in composted sludge after application to soil. Chemosphere. 2013 Aug; 92(10):1280-5.
Not within scope/ecological and fate	Qin J, Li H, Lin C, Chen G	2013	Qin J, Li H, Lin C, Chen G.Can rainwater induce Fenton-driven degradation of herbicides in natural waters? Chemosphere. 2013 Aug; 92(8):1048-52.
Not within scope/ecological and fate	Li W, Wang YJ, Zhu M, Fan TT, Zhou DM, Phillips BL, Sparks DL	2013	Li W, Wang YJ, Zhu M, Fan TT, Zhou DM, Phillips BL, Sparks DL.Inhibition mechanisms of Zn precipitation on aluminum oxide by glyphosate: a 31P NMR and Zn EXAFS study. Environ Sci Technol. 2013 May 7; 47(9):4211-9.
Formulation/no glyphosate measurements	Jasper R, Locatelli GO, Pilati C, Locatelli C	2012	Jasper R, Locatelli GO, Pilati C, Locatelli C.Evaluation of biochemical, hematological and oxidative parameters in mice exposed to the herbicide glyphosate-Roundup(Â®). Interdiscip Toxicol. 2012 Sep; 5(3):133-40.
Not within scope/ methods generating	Cao L, Liang S, Tan X, Meng J	2012	Cao L, Liang S, Tan X, Meng J.[Capillary electrophoresis analysis for glyphosate, glufosinate and aminomethylphosphonic acid with laser-induced fluorescence detection]. Se Pu. 2012 Dec; 30(12):1295-300.
Not within scope/ methods generating	da Silva ER, Segato TP, Coltro WK, Lima RS, Carrilho E, Mazo LH	2013	da Silva ER, Segato TP, Coltro WK, Lima RS, Carrilho E, Mazo LH.Determination of glyphosate and AMPA on polyester-toner electrophoresis microchip with contactless conductivity detection. Electrophoresis. 2013 Jul; 34(14):2107-11.
Not within scope/ecological and fate	Liu YB, Long MX, Yin YJ, Si MR, Zhang L, Lu ZQ, Wang Y, Shen XH	2013	Liu YB, Long MX, Yin YJ, Si MR, Zhang L, Lu ZQ, Wang Y, Shen XH.Physiological roles of mycothiol in detoxification and tolerance to multiple poisonous chemicals in Corynebacterium glutamicum. Arch Microbiol. 2013 Jun; 195(6):419-29.
Not within scope/ecological and fate	UlÃ©n BM, Larsbo M, Kreuger JK, SvanbÃ¤ck A	2014	UlÃ©n BM, Larsbo M, Kreuger JK, SvanbÃ¤ck A.Spatial variation in herbicide leaching from a marine clay soil via subsurface drains. Pest Manag Sci. 2014 Mar; 70(3):405-14.

Comments	Authors	PubDate (Year)	Full Citation
Not within scope/ecological and fate	Daouk S, Copin PJ, Rossi L, ChÃ¨vre N, Pfeifer HR	2013	Daouk S, Copin PJ, Rossi L, ChÃ¨vre N, Pfeifer HR.Dynamics and environmental risk assessment of the herbicide glyphosate and its metabolite AMPA in a small vineyard river of the Lake Geneva catchment. Environ Toxicol Chem. 2013 Sep; 32(9):2035-44.
Epidemiology	Henneberger PK, Liang X, London SJ, Umbach DM, Sandler DP, Hoppin JA	2014	Henneberger PK, Liang X, London SJ, Umbach DM, Sandler DP, Hoppin JA.Exacerbation of symptoms in agricultural pesticide applicators with asthma. Int Arch Occup Environ Health. 2014 May; 87(4):423-32.
Not within scope/ecological and fate	Lashermes G, Zhang Y, Houot S, Steyer JP, Patureau D, Barriuso E, Garnier P	2013	Lashermes G, Zhang Y, Houot S, Steyer JP, Patureau D, Barriuso E, Garnier P.Simulation of Organic Matter and Pollutant Evolution during Composting: The COP-Compost Model. J Environ Qual. 2013 Mar-Apr; 42(2):361-72.
Not within scope/ methods generating	Ding X, Yang KL	2013	Ding X, Yang KL.Development of an oligopeptide functionalized surface plasmon resonance biosensor for online detection of glyphosate. Anal Chem. 2013 Jun 18; 85(12):5727-33.
Not within scope/ecological and fate	Daouk S, Grandjean D, Chevre N, De Alencastro LF, Pfeifer HR	2013	Daouk S, Grandjean D, Chevre N, De Alencastro LF, Pfeifer HR.The herbicide glyphosate and its metabolite AMPA in the Lavaux vineyard area, Western Switzerland: proof of widespread export to surface waters. Part I: method validation in different water matrices. J Environ Sci Health B. 2013; 48(9):717-24.
Not within scope/ecological and fate	Daouk S, De Alencastro LF, Pfeifer HR	2013	Daouk S, De Alencastro LF, Pfeifer HR.The herbicide glyphosate and its metabolite AMPA in the Lavaux vineyard area, western Switzerland: proof of widespread export to surface waters. Part II: the role of infiltration and surface runoff. J Environ Sci Health B. 2013; 48(9):725-36.
Not within scope/ecological and fate	Zhou Y, Guo Y, Xu S, Zhang L, Ahmad W, Shi Z	2013	Zhou Y, Guo Y, Xu S, Zhang L, Ahmad W, Shi Z.Photoluminescent 3D lanthanide-organic frameworks based on 2,5-dioxo-1,4-piperazinylbis(methylphosphonic) acid formed via in situ cyclodehydration of glyphosates. Inorg Chem. 2013 Jun 3; 52(11):6338-45.
Not within scope/ecological and fate	Zhou CF, Wang YJ, Li CC, Sun RJ, Yu YC, Zhou DM	2013	Zhou CF, Wang YJ, Li CC, Sun RJ, Yu YC, Zhou DM.Subacute toxicity of copper and glyphosate and their interaction toÂ earthworm (Eisenia fetida). Environ Pollut. 2013 Sep; 180:71-7.

Comments	Authors	PubDate (Year)	Full Citation
In vitro/no glyphosate measurements	Thongprakaisang S, Thiantanawat A, Rangkadilok N, Suriyo T, Satayavivad J	2013	Thongprakaisang S, Thiantanawat A, Rangkadilok N, Suriyo T, Satayavivad J.Glyphosate induces human breast cancer cells growth via estrogen receptors. Food Chem Toxicol. 2013 Sep; 59:129-36.
Not within scope/ecological and fate	Iummato MM, Di Fiori E, Sabatini SE, Cacciatore LC, CochÃ³n AC, de Molina Mdel C, JuÃ¡rez AB	2013	Iummato MM, Di Fiori E, Sabatini SE, Cacciatore LC, CochÃ³n AC, de Molina Mdel C, JuÃ¡rez AB.Evaluation of biochemical markers in the golden mussel Limnoperna fortunei exposed to glyphosate acid in outdoor microcosms. Ecotoxicol Environ Saf. 2013 Sep; 95:123-9.
Not within scope/ecological and fate	Chennappa G, Adkar-Purushothama CR, Suraj U, Tamilvendan K, Sreenivasa MY	2014	Chennappa G, Adkar-Purushothama CR, Suraj U, Tamilvendan K, Sreenivasa MY.Pesticide tolerant Azotobacter isolates from paddy growing areas of northern Karnataka, India. World J Microbiol Biotechnol. 2014 Jan; 30(1):1-7.
Not within scope/ exposure and monitoring	Espanhol-Soares M, Nociti LA, Machado-Neto JG	2013	Espanhol-Soares M, Nociti LA, Machado-Neto JG.Procedures to evaluate the efficiency of protective clothing worn by operators applying pesticide. Ann Occup Hyg. 2013 Oct; 57(8):1041-53.
Not within scope/ecological and fate	Nwani CD, Nagpure NS, Kumar R, Kushwaha B, Lakra WS	2013	Nwani CD, Nagpure NS, Kumar R, Kushwaha B, Lakra WS.DNA damage and oxidative stress modulatory effects of glyphosate-based herbicide in freshwater fish, Channa punctatus. Environ Toxicol Pharmacol. 2013 Sep; 36(2):539-47.
Formulation/no glyphosate measurements	de Liz Oliveira Cavalli VL, Cattani D, Heinz Rieg CE, Pierozan P, Zanatta L, Benedetti Parisotto E, Wilhelm Filho D, Mena Barreto Silva FR, Pessoa-Pureur R, Zamoner A	2013	de Liz Oliveira Cavalli VL, Cattani D, Heinz Rieg CE, Pierozan P, Zanatta L, Benedetti Parisotto E, Wilhelm Filho D, Mena Barreto Silva FR, Pessoa-Pureur R, Zamoner A.Roundup disrupts male reproductive functions by triggering calcium-mediated cell death in rat testis and Sertoli cells. Free Radic Biol Med. 2013 Dec; 65:335-46.
Not within scope/ methods generating	Shen J, Huang J, Liu L, Ye W, Lin J, Van der Bruggen B	2013	Shen J, Huang J, Liu L, Ye W, Lin J, Van der Bruggen B.The use of BMED for glyphosate recovery from glyphosate neutralization liquor in view of zero discharge. J Hazard Mater. 2013 Sep 15; 260:660-7.
Not within scope/ecological and fate	Aparicio VC, De GerÃ³nimo E, Marino D, Primost J, Carriquiriborde P, Costa JL	2013	Aparicio VC, De GerÃ³nimo E, Marino D, Primost J, Carriquiriborde P, Costa JL.Environmental fate of glyphosate and aminomethylphosphonic acid in surface waters and soil of agricultural basins. Chemosphere. 2013 Nov; 93(9):1866-73.

Comments	Authors	PubDate (Year)	Full Citation
Epidemiology	Dawson AH, Wilks MF	2013	Dawson AH, Wilks MF.With the benefit of hindsight: trials using retrospective controls versus randomized controlled trials in clinical toxicology. Clin Toxicol (Phila). 2013 Aug; 51(7):525-6.
Not within scope/ecological and fate	Magbanua FS, Townsend CR, Hageman KJ, Lange K, Lear G, Lewis GD, Matthaei CD	2013	Magbanua FS, Townsend CR, Hageman KJ, Lange K, Lear G, Lewis GD, Matthaei CD.Understanding the combined influence of fine sediment and glyphosate herbicide on stream periphyton communities. Water Res. 2013 Sep 15; 47(14):5110-20.
Poisoning or incident	Gil HW, Park JS, Park SH, Hong SY	2013	Gil HW, Park JS, Park SH, Hong SY.Effect of intravenous lipid emulsion in patients with acute glyphosate intoxication. Clin Toxicol (Phila). 2013 Sep-Oct; 51(8):767-71.
Not within scope/ecological and fate	Boonsoong B, Bullangpoti V	2012	Boonsoong B, Bullangpoti V.Acute toxicity of Roundup and carbosulfan to the Thai fairy shrimp, Branchinella thailandensis. Commun Agric Appl Biol Sci. 2012; 77(4):431-7.
Not within scope/ecological and fate	Sandy EH, Blake RE, Chang SJ, Jun Y, Yu C	2013	Sandy EH, Blake RE, Chang SJ, Jun Y, Yu C.Oxygen isotope signature of UV degradation of glyphosate and phosphonoacetate: tracing sources and cycling of phosphonates. J Hazard Mater. 2013 Sep 15; 260:947-54.
Not within scope/ecological and fate	Zhidenko AA, Bibchuk EV, Barbukho EV	2013	Zhidenko AA, Bibchuk EV, Barbukho EV.[Effect of glyphosate on the energy exchange in carp organs]. Ukr Biokhim Zh (1999). 2013 May-Jun; 85(3):22-30.
Not within scope/ecological and fate	MalÃ©cot M, GuÃ©vel B, Pineau C, Holbech BF, Bormans M, Wiegand C	2013	MalÃ©cot M, GuÃ©vel B, Pineau C, Holbech BF, Bormans M, Wiegand C.Specific proteomic response of Unio pictorum mussel to a mixture of glyphosate and microcystin-LR. J Proteome Res. 2013 Nov 1; 12(11):5281-92.
Treatment/In vitro/no glyphosate measurements/glyphosate and AMPA inhibited cancer cell growth and no effect on normal cells	Li Q, Lambrechts MJ, Zhang Q, Liu S, Ge D, Yin R, Xi M, You Z	2013	Li Q, Lambrechts MJ, Zhang Q, Liu S, Ge D, Yin R, Xi M, You Z.Glyphosate and AMPA inhibit cancer cell growth through inhibiting intracellular glycine synthesis. Drug Des Devel Ther. 2013; 7:635-43.

Comments	Authors	PubDate (Year)	Full Citation
Poisoning or incident	Chen HH, Lin JL, Huang WH, Weng CH, Lee SY, Hsu CW, Chen KH, Wang IK, Liang CC, Chang CT, Yen TH	2013	Chen HH, Lin JL, Huang WH, Weng CH, Lee SY, Hsu CW, Chen KH, Wang IK, Liang CC, Chang CT, Yen TH.Spectrum of corrosive esophageal injury after intentional paraquat or glyphosate-surfactant herbicide ingestion. Int J Gen Med. 2013; 6:677-83.
Not within scope/ecological and fate	Vera-Candioti J, Soloneski S, Larramendy ML	2013	Vera-Candioti J, Soloneski S, Larramendy ML.Single-cell gel electrophoresis assay in the ten spotted live-bearer fish, Cnesterodon decemmaculatus (Jenyns, 1842), as bioassay for agrochemical-induced genotoxicity. Ecotoxicol Environ Saf. 2013 Dec; 98:368-73.
Not within scope/ exposure and monitoring	Stewart M, Olsen G, Hickey CW, Ferreira B, JeliÄ‡ A, PetroviÄ‡ M, Barcelo D	2014	Stewart M, Olsen G, Hickey CW, Ferreira B, JeliÄ‡ A, PetroviÄ‡ M, Barcelo D.A survey of emerging contaminants in the estuarine receiving environment around Auckland, New Zealand. Sci Total Environ. 2014 Jan 15; 468-469:202-10.
Not within scope/ecological and fate	Harayashiki CA, Varela AS Jr, Machado AA, Cabrera Lda C, Primel EG, Bianchini A, Corcini CD	2013	Harayashiki CA, Varela AS Jr, Machado AA, Cabrera Lda C, Primel EG, Bianchini A, Corcini CD.Toxic effects of the herbicide Roundup in the guppy Poecilia vivipara acclimated to fresh water. Aquat Toxicol. 2013 Oct 15; 142-143:176-84.
Not within scope/ecological and fate	De Souza Filho J, Sousa CC, Da Silva CC, De SabÃ³ia-Morais SM, Grisolia CK	2013	De Souza Filho J, Sousa CC, Da Silva CC, De SabÃ³ia-Morais SM, Grisolia CK.Mutagenicity and genotoxicity in gill erythrocyte cells of Poecilia reticulata exposed to a glyphosate formulation. Bull Environ Contam Toxicol. 2013 Nov; 91(5):583-7.
Not within scope/ methods generating	MartÃnez Gil P, Laguarda-Miro N, Camino JS, Peris RM	2013	MartÃnez Gil P, Laguarda-Miro N, Camino JS, Peris RM.Glyphosate detection with ammonium nitrate and humic acids as potential interfering substances by pulsed voltammetry technique. Talanta. 2013 Oct 15; 115:702-5.
In vitro/no glyphosate measurements	George J, Shukla Y	2013	George J, Shukla Y.Emptying of Intracellular Calcium Pool and Oxidative Stress Imbalance Are Associated with the Glyphosate-Induced Proliferation in Human Skin Keratinocytes HaCaT Cells. ISRN Dermatol. 2013; 2013:825180.
Not within scope/ecological and fate	Taddei M, Donnadio A, Costantino F, Vivani R, Casciola M	2013	Taddei M, Donnadio A, Costantino F, Vivani R, Casciola M.Synthesis, crystal structure, and proton conductivity of one-dimensional, two-dimensional, and three-dimensional zirconium phosphonates based on glyphosate and glyphosine. Inorg Chem. 2013 Oct 21; 52(20):12131-9.

Comments	Authors	PubDate (Year)	Full Citation
Not within scope/ecological and fate	Gholami-Seyedkolaei SJ, Mirvaghefi A, Farahmand H, Kosari AA	2013	Gholami-Seyedkolaei SJ, Mirvaghefi A, Farahmand H, Kosari AA.Effect of a glyphosate-based herbicide in Cyprinus carpio: assessment of acetylcholinesterase activity, hematological responses and serum biochemical parameters. Ecotoxicol Environ Saf. 2013 Dec; 98:135-41.
Not within scope/ecological and fate	Gholami-Seyedkolaei SJ, Mirvaghefi A, Farahmand H, Kosari AA, Gholami-Seyedkolaei SJ, Gholami-Seyedkolaei SJ	2013	Gholami-Seyedkolaei SJ, Mirvaghefi A, Farahmand H, Kosari AA, Gholami-Seyedkolaei SJ, Gholami-Seyedkolaei SJ.Optimization of recovery patterns in common carp exposed to roundup using response surface methodology: evaluation of neurotoxicity and genotoxicity effects and biochemical parameters. Ecotoxicol Environ Saf. 2013 Dec; 98:152-61.
Not within scope/ecological and fate	Hedegaard MJ, Albrechtsen HJ	2014	Hedegaard MJ, Albrechtsen HJ.Microbial pesticide removal in rapid sand filters for drinking water treatment--potential and kinetics. Water Res. 2014 Jan 1; 48:71-81.
Not within scope/ecological and fate	Rittman S, Wrinn KM, Evans SC, Webb AW, Rypstra AL	2013	Rittman S, Wrinn KM, Evans SC, Webb AW, Rypstra AL.Glyphosate-based herbicide has contrasting effects on prey capture by two co-occurring wolf spider species. J Chem Ecol. 2013 Oct; 39(10):1247-53.
Editorial	Bates N, Edwards N	2013	Bates N, Edwards N.Glyphosate toxicity in animals. Clin Toxicol (Phila). 2013 Dec; 51(10):1243.
Not within scope/ exposure and monitoring	Zhang RN, Liu HL, Huo ZL, Zhang F, Ma YJ, Zhu BL, Dou JR, Mao YY	2013	Zhang RN, Liu HL, Huo ZL, Zhang F, Ma YJ, Zhu BL, Dou JR, Mao YY.[Determination of glyphosate in air of workplaces by ion chromatography]. Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi. 2013 Oct; 31(10):779-82.
Not within scope/ exposure and monitoring	Tush D, Loftin KA, Meyer MT	2013	Tush D, Loftin KA, Meyer MT.Characterization of polyoxyethylene tallow amine surfactants in technical mixtures and glyphosate formulations using ultra-high performance liquid chromatography and triple quadrupole mass spectrometry. J Chromatogr A. 2013 Dec 6; 1319:80-7.
Not within scope/ exposure and monitoring	Van Stempvoort DR, Roy JW, Brown SJ, Bickerton G	2014	Van Stempvoort DR, Roy JW, Brown SJ, Bickerton G.Residues of the herbicide glyphosate in riparian groundwater in urban catchments. Chemosphere. 2014 Jan; 95:455-63.
Not within scope/ecological and fate	Omran NE, Salama WM	2013	Omran NE, Salama WM.The endocrine disrupter effect of atrazine and glyphosate on Biomphalaria alexandrina snails. Toxicol Ind Health. 2013 Nov 8;

Comments	Authors	PubDate (Year)	Full Citation
Not within scope/ecological and fate	FernÃ¡ndez L, de Haro LA, Distefano AJ, Carolina MartÃnez M, LÃa V, Papa JC, Olea I, Tosto D, Esteban Hopp H	2013	FernÃ¡ndez L, de Haro LA, Distefano AJ, Carolina MartÃnez M, LÃa V, Papa JC, Olea I, Tosto D, Esteban Hopp H.Population genetics structure of glyphosate-resistant Johnsongrass (Sorghum halepense L. Pers) does not support a single origin of the resistance. Ecol Evol. 2013 Sep; 3(10):3388-400.
Not within scope/ecological and fate	Zhan T, Zhang K, Chen Y, Lin Y, Wu G, Zhang L, Yao P, Shao Z, Liu Z	2013	Zhan T, Zhang K, Chen Y, Lin Y, Wu G, Zhang L, Yao P, Shao Z, Liu Z.Improving glyphosate oxidation activity of glycine oxidase from Bacillus cereus by directed evolution. PLoS One. 2013; 8(11):e79175.
In vitro/formulation/no glyphosate measurements	Shehata AA, KÃ¼hnert M, Haufe S, KrÃ¼ger M	2014	Shehata AA, KÃ¼hnert M, Haufe S, KrÃ¼ger M.Neutralization of the antimicrobial effect of glyphosate by humic acid in vitro. Chemosphere. 2014 Jun; 104:258-61.
Not available in English	Zhao W, Yu H, Zhang J, Shu L	2013	Zhao W, Yu H, Zhang J, Shu L.[Effects of glyphosate on apoptosis and expressions of androgen-binding protein and vimentin mRNA in mouse Sertoli cells]. Nan Fang Yi Ke Da Xue Xue Bao. 2013 Nov; 33(11):1709-13.
Not within scope/ methods generating	Mack CM, Lin BJ, Turner JD, Johnstone AF, Burgoon LD, Shafer TJ	2014	Mack CM, Lin BJ, Turner JD, Johnstone AF, Burgoon LD, Shafer TJ.Burst and principal components analyses of MEA data for 16 chemicals describe at least three effects classes. Neurotoxicology. 2014 Jan; 40:75-85.
Not within scope/ecological and fate	Nwani CD, Nagpure NS, Kumar R, Kushwaha B, Kumar P, Lakra WS	2014	Nwani CD, Nagpure NS, Kumar R, Kushwaha B, Kumar P, Lakra WS.Induction of micronuclei and nuclear lesions in Channa punctatus following exposure to carbosulfan, glyphosate and atrazine. Drug Chem Toxicol. 2014 Oct; 37(4):370-7.
Not within scope/ methods generating	Zhu L, Zhang J, Guo Y	2014	Zhu L, Zhang J, Guo Y.Enhanced detection and desalting free protocol for phosphopeptides eluted from immobilized Fe (III) affinity chromatography in direct MALDI TOF analysis. J Proteomics. 2014 Jan 16; 96:360-5.
Not within scope/ecological and fate	Cao G, Liu Y, Liu G, Wang J, Wang G	2013	Cao G, Liu Y, Liu G, Wang J, Wang G.Draft genome sequence of pseudomonas strain p818, isolated from glyphosate-polluted soil. Genome Announc. 2013 Dec 19; 1(6)
Poisoning or incident	Kim YH, Lee JH, Hong CK, Cho KW, Park YH, Kim YW, Hwang SY	2014	Kim YH, Lee JH, Hong CK, Cho KW, Park YH, Kim YW, Hwang SY.Heart rate-corrected QT interval predicts mortality in glyphosate-surfactant herbicide-poisoned patients. Am J Emerg Med. 2014 Mar; 32(3):203-7.

Comments	Authors	PubDate (Year)	Full Citation
Model application	Wunnapuk K, Gobe G, Endre Z, Peake P, Grice JE, Roberts MS, Buckley NA, Liu X	2014	Wunnapuk K, Gobe G, Endre Z, Peake P, Grice JE, Roberts MS, Buckley NA, Liu X.Use of a glyphosate-based herbicide-induced nephrotoxicity model to investigate a panel of kidney injury biomarkers. Toxicol Lett. 2014 Feb 10; 225(1):192-200.
Not within scope/ecological and fate	Uren Webster TM, Laing LV, Florance H, Santos EM	2014	Uren Webster TM, Laing LV, Florance H, Santos EM.Effects of glyphosate and its formulation, roundup, on reproduction in zebrafish (Danio rerio). Environ Sci Technol. 2014 Jan 21; 48(2):1271-9.
Poisoning or incident	Garlich FM, Goldman M, Pepe J, Nelson LS, Allan MJ, Goldstein DA, Goldfarb DS, Hoffman RS	2014	Garlich FM, Goldman M, Pepe J, Nelson LS, Allan MJ, Goldstein DA, Goldfarb DS, Hoffman RS.Hemodialysis clearance of glyphosate following a life-threatening ingestion of glyphosate-surfactant herbicide. Clin Toxicol (Phila). 2014 Jan; 52(1):66-71.
Not relevant	Dai X, Zhu M, Wang YP	2014	Dai X, Zhu M, Wang YP.Circular permutation of E. coli EPSP synthase: increased inhibitor resistance, improved catalytic activity, and an indicator for protein fragment complementation. Chem Commun (Camb). 2014 Feb 21; 50(15):1830-2.
Not within scope/ecological and fate	Richard S, PrÃ©vot-D'Alvise N, Bunet R, Simide R, Couvray S, CoupÃ© S, Grillasca JP	2014	Richard S, PrÃ©vot-D'Alvise N, Bunet R, Simide R, Couvray S, CoupÃ© S, Grillasca JP.Effect of a glyphosate-based herbicide on gene expressions of the cytokines interleukin-1Î² and interleukin-10 and of heme oxygenase-1 in European sea bass, Dicentrarchus labrax L. Bull Environ Contam Toxicol. 2014 Mar; 92(3):294-9.
Not within scope/ exposure and monitoring	Ramwell CT, Kah M, Johnson PD	2014	Ramwell CT, Kah M, Johnson PD.Contribution of household herbicide usage to glyphosate and its degradate aminomethylphosphonic acid in surface water drains. Pest Manag Sci. 2014 Dec; 70(12):1823-30.
Not within scope/ecological and fate	Mysore Doddaiah K, Narayan A, Gokare Aswathanarayana R, Ravi S	2013	Mysore Doddaiah K, Narayan A, Gokare Aswathanarayana R, Ravi S.Effect of metabolic inhibitors on growth and carotenoid production in Dunaliella bardawil. J Food Sci Technol. 2013 Dec; 50(6):1130-6.
Not within scope/ecological and fate	Carranza CS, Bergesio MV, Barberis CL, Chiacchiera SM, Magnoli CE	2014	Carranza CS, Bergesio MV, Barberis CL, Chiacchiera SM, Magnoli CE.Survey of Aspergillus section Flavi presence in agricultural soils and effect of glyphosate on nontoxigenic A. flavus growth on soil-based medium. J Appl Microbiol. 2014 May; 116(5):1229-40.

Comments	Authors	PubDate (Year)	Full Citation
Not within scope/ecological and fate	dos Santos KC, Martinez CB	2014	dos Santos KC, Martinez CB.Genotoxic and biochemical effects of atrazine and Roundup(Â®), alone and in combination, on the Asian clam Corbicula fluminea. Ecotoxicol Environ Saf. 2014 Feb; 100:7-14.
In vitro/no glyphosate measurements	Chaufan G, Coalova I, RÃos de Molina Mdel C	2014	Chaufan G, Coalova I, RÃos de Molina Mdel C.Glyphosate commercial formulation causes cytotoxicity, oxidative effects, and apoptosis on human cells: differences with its active ingredient. Int J Toxicol. 2014 Jan-Feb; 33(1):29-38.
Not within scope/ecological and fate	Moreno NC, Sofia SH, Martinez CB	2014	Moreno NC, Sofia SH, Martinez CB.Genotoxic effects of the herbicide Roundup Transorb and its active ingredient glyphosate on the fish Prochilodus lineatus. Environ Toxicol Pharmacol. 2014 Jan; 37(1):448-54.
Not within scope/ecological and fate	Prevot-D'Alvise N, Richard S, CoupÃ© S, Bunet R, Grillasca JP	2013	Prevot-D'Alvise N, Richard S, CoupÃ© S, Bunet R, Grillasca JP.Acute toxicity of a commercial glyphosate formulation on European sea bass juveniles (Dicentrarchus labrax L.): gene expressions of heme oxygenase-1 (ho-1), acetylcholinesterase (AChE) and aromatases (cyp19a and cyp19b). Cell Mol Biol (Noisy-le-grand). 2013 Dec 31; 59 Suppl:OL1906-17.
Not within scope/ecological and fate	Mbanaso FU, Coupe SJ, Charlesworth SM, Nnadi EO, Ifelebuegu AO	2014	Mbanaso FU, Coupe SJ, Charlesworth SM, Nnadi EO, Ifelebuegu AO.Potential microbial toxicity and non-target impact of different concentrations of glyphosate-containing herbicide (GCH) in a model Pervious Paving System. Chemosphere. 2014 Apr; 100:34-41.
Not within scope/ecological and fate	Mercurio P, Flores F, Mueller JF, Carter S, Negri AP	2014	Mercurio P, Flores F, Mueller JF, Carter S, Negri AP.Glyphosate persistence in seawater. Mar Pollut Bull. 2014 Aug 30; 85(2):385-90.
Only abstract English	Kwiatkowska M, PaweÅ, J, Bukowska B	2013	Kwiatkowska M, PaweÅ, J, Bukowska B.[Glyphosate and its formulations--toxicity, occupational and environmental exposure]. Med Pr. 2013; 64(5):717-29.
Not within scope/ecological and fate	Okonkwo FO, Ejike CE, Anoka AN, Onwurah IN	2013	Okonkwo FO, Ejike CE, Anoka AN, Onwurah IN.Toxicological studies on the short term exposure of Clarias albopunctatus (Lamonte and Nichole 1927) to sub-lethal concentrations of roundup. Pak J Biol Sci. 2013 Sep 15; 16(18):939-44.
Not within scope/ecological and fate	Bayir M, Sirkecioglu AN, Bayir A, Aras M	2013	Bayir M, Sirkecioglu AN, Bayir A, Aras M.Alterations in fatty acids of polar lipids in Salmo trutta on long-term exposure to a glyphosate-based herbicide (Roundup). Pak J Biol Sci. 2013 Oct 15; 16(20):1194-8.

Comments	Authors	PubDate (Year)	Full Citation
Not within scope/ methods generating	Mol HG, van Dam RC	2014	Mol HG, van Dam RC.Rapid detection of pesticides not amenable to multi-residue methods by flow injection-tandem mass spectrometry. Anal Bioanal Chem. 2014 Nov; 406(27):6817-25.
Not within scope/ecological and fate	Mujahid M, Sasikala Ch, Ramana ChV	2014	Mujahid M, Sasikala Ch, Ramana ChV.Aniline is an inducer, and not a precursor, for indole derivatives in Rubrivivax benzoatilyticus JA2. PLoS One. 2014; 9(2):e87503.
Not within scope/ecological and fate	Hock SD, Poulin R	2012	Hock SD, Poulin R.Exposure of the snail Potamopyrgus antipodarum to herbicide boosts output and survival of parasite infective stages. Int J Parasitol Parasites Wildl. 2012 Dec; 1:13-8.
Not within scope/ hypothesis generating	Jayasumana C, Gunatilake S, Senanayake P	2014	Jayasumana C, Gunatilake S, Senanayake P.Glyphosate, hard water and nephrotoxic metals: are they the culprits behind the epidemic of chronic kidney disease of unknown etiology in Sri Lanka? Int J Environ Res Public Health. 2014 Feb 20; 11(2):2125-47.
In vitro/no concentration measurements for glyphosate, metabolites, or impurities	Kwiatkowska M, Huras B, Bukowska B	2014	Kwiatkowska M, Huras B, Bukowska B.The effect of metabolites and impurities of glyphosate on human erythrocytes (in vitro). Pestic Biochem Physiol. 2014 Feb; 109:34-43.
Not within scope/ecological and fate	Avigliano L, Fassiano AV, Medesani DA, RÃos de Molina MC, RodrÃguez EM	2014	Avigliano L, Fassiano AV, Medesani DA, RÃos de Molina MC, RodrÃguez EM.Effects of glyphosate on growth rate, metabolic rate and energy reserves of early juvenile crayfish, Cherax quadricarinatus M. Bull Environ Contam Toxicol. 2014 Jun; 92(6):631-5.
Not within scope/ecological and fate	Hove-Jensen B, Zechel DL, Jochimsen B	2014	Hove-Jensen B, Zechel DL, Jochimsen B.Utilization of glyphosate as phosphate source: biochemistry and genetics of bacterial carbon-phosphorus lyase. Microbiol Mol Biol Rev. 2014 Mar; 78(1):176-97.
Not within scope/ecological and fate	Annett R, Habibi HR, Hontela A	2014	Annett R, Habibi HR, Hontela A.Impact of glyphosate and glyphosate-based herbicides on the freshwater environment. J Appl Toxicol. 2014 May; 34(5):458-79.

Comments	Authors	PubDate (Year)	Full Citation
Not within scope/ecological and fate	Armiliato N, Ammar D, Nezzi L, Straliotto M, Muller YM, Nazari EM	2014	Armiliato N, Ammar D, Nezzi L, Straliotto M, Muller YM, Nazari EM.Changes in ultrastructure and expression of steroidogenic factor-1 in ovaries of zebrafish Danio rerio exposed to glyphosate. J Toxicol Environ Health A. 2014; 77(7):405-14.
Not within scope/ecological and fate	Yang C, Shen S, Wang M, Li J	2013	Yang C, Shen S, Wang M, Li J.Mild salinization stimulated glyphosate degradation and microbial activities in a riparian soil from Chongming Island, China. J Environ Biol. 2013 Apr; 34(2 Spec No):367-73.
Formulation/no glyphosate measurements	Cattani D, de Liz Oliveira Cavalli VL, Heinz Rieg CE, Domingues JT, Dal-Cim T, Tasca CI, Mena Barreto Silva FR, Zamoner A	2014	Cattani D, de Liz Oliveira Cavalli VL, Heinz Rieg CE, Domingues JT, Dal-Cim T, Tasca CI, Mena Barreto Silva FR, Zamoner A.Mechanisms underlying the neurotoxicity induced by glyphosate-based herbicide in immature rat hippocampus: involvement of glutamate excitotoxicity. Toxicology. 2014 Jun 5; 320:34-45.
Not relevant	Oh JS, Choi KH	2014	Oh JS, Choi KH.Methemoglobinemia associated with metaflumizone poisoning. Clin Toxicol (Phila). 2014 Apr; 52(4):288-90.
Review	Samsel A, Seneff S	2013	Samsel A, Seneff S.Glyphosate, pathways to modern diseases II: Celiac sprue and gluten intolerance. Interdiscip Toxicol. 2013 Dec; 6(4):159-84.
In vitro/no glyphosate measurements	Alvarez-Moya C, Silva MR, RamÃrez CV, Gallardo DG, SÃ¡nchez RL, Aguirre AC, Velasco AF	2014	Alvarez-Moya C, Silva MR, RamÃrez CV, Gallardo DG, SÃ¡nchez RL, Aguirre AC, Velasco AF.Comparison of the in vivo and in vitro genotoxicity of glyphosate isopropylamine salt in three different organisms. Genet Mol Biol. 2014 Mar; 37(1):105-10.
Not within scope/ecological and fate	Guilherme S, Santos MA, GaivÃ£o I, Pacheco M	2014	Guilherme S, Santos MA, GaivÃ£o I, Pacheco M.DNA and chromosomal damage induced in fish (Anguilla anguilla L.) by aminomethylphosphonic acid (AMPA)--the major environmental breakdown product of glyphosate. Environ Sci Pollut Res Int. 2014; 21(14):8730-9.
Not within scope/ methods generating	Prasad BB, Jauhari D, Tiwari MP	2014	Prasad BB, Jauhari D, Tiwari MP.Doubly imprinted polymer nanofilm-modified electrochemical sensor for ultra-trace simultaneous analysis of glyphosate and glufosinate. Biosens Bioelectron. 2014 Sep 15; 59:81-8.
In vitro/no glyphosate measurements	Mesnage R, Defarge N, Spiroux de VendÃ´mois J, SÃ©ralini GE	2014	Mesnage R, Defarge N, Spiroux de VendÃ´mois J, SÃ©ralini GE.Major pesticides are more toxic to human cells than their declared active principles. Biomed Res Int. 2014; 2014:179691.

Comments	Authors	PubDate (Year)	Full Citation
Not within scope/ecological and fate	Helmer SH, Kerbaol A, Aras P, Jumarie C, Boily M	2015	Helmer SH, Kerbaol A, Aras P, Jumarie C, Boily M.Effects of realistic doses of atrazine, metolachlor, and glyphosate on lipid peroxidation and diet-derived antioxidants in caged honey bees (Apis mellifera). Environ Sci Pollut Res Int. 2015 Jun; 22(11):8010-21.
Not within scope/ecological and fate	Yusof S, Ismail A, Alias MS	2014	Yusof S, Ismail A, Alias MS.Effect of glyphosate-based herbicide on early life stages of Java medaka (Oryzias javanicus): a potential tropical test fish. Mar Pollut Bull. 2014 Aug 30; 85(2):494-8.
Poisoning or incident	Perry L, Adams RD, Bennett AR, Lupton DJ, Jackson G, Good AM, Thomas SH, Vale JA, Thompson JP, Bateman DN, Eddleston M	2014	Perry L, Adams RD, Bennett AR, Lupton DJ, Jackson G, Good AM, Thomas SH, Vale JA, Thompson JP, Bateman DN, Eddleston M.National toxicovigilance for pesticide exposures resulting in health care contact - An example from the UK's National Poisons Information Service. Clin Toxicol (Phila). 2014 Jun; 52(5):549-55.
Not within scope/ecological and fate	Aslam S, Iqbal A, Deschamps M, Recous S, Garnier P, Benoit P	2015	Aslam S, Iqbal A, Deschamps M, Recous S, Garnier P, Benoit P.Effect of rainfall regimes and mulch decomposition on the dissipation and leaching of S-metolachlor and glyphosate: a soil column experiment. Pest Manag Sci. 2015 Feb; 71(2):278-91.
Editorial	Campbell AW	2014	Campbell AW.Glyphosate: its effects on humans. Altern Ther Health Med. 2014 May-Jun; 20(3):9-11.
Review	Schinasi L, Leon ME	2014	Schinasi L, Leon ME.Non-Hodgkin lymphoma and occupational exposure to agricultural pesticide chemical groups and active ingredients: a systematic review and meta-analysis. Int J Environ Res Public Health. 2014 Apr 23; 11(4):4449-527.
In vitro/no measurements of glyphosate, metabolites, or impurities	Kwiatkowska M, Nowacka-Krukowska H, Bukowska B	2014	Kwiatkowska M, Nowacka-Krukowska H, Bukowska B.The effect of glyphosate, its metabolites and impurities on erythrocyte acetylcholinesterase activity. Environ Toxicol Pharmacol. 2014 May; 37(3):1101-8.
Not within scope/ecological and fate	Saunders LE, Koontz MB, Pezeshki R	2013	Saunders LE, Koontz MB, Pezeshki R.Root-zone glyphosate exposure adversely affects two ditch species. Biology (Basel). 2013 Dec 18; 2(4):1488-96.

Comments	Authors	PubDate (Year)	Full Citation
Not within scope/ecological and fate	Selvi AA, Manonmani HK	2015	Selvi AA, Manonmani HK.Purification and characterization of carbon-phosphorus bond-cleavage enzyme from glyphosate degrading Pseudomonas putida T5. Prep Biochem Biotechnol. 2015; 45(4):380-97.
Not within scope/ecological and fate	Sinhorin VD, Sinhorin AP, Teixeira JM, MilÃ©ski KM, Hansen PC, Moreira PS, Kawashita NH, Baviera AM, Loro VL	2014	Sinhorin VD, Sinhorin AP, Teixeira JM, MilÃ©ski KM, Hansen PC, Moreira PS, Kawashita NH, Baviera AM, Loro VL.Effects of the acute exposition to glyphosate-based herbicide on oxidative stress parameters and antioxidant responses in a hybrid Amazon fish surubim (Pseudoplatystoma sp). Ecotoxicol Environ Saf. 2014 Aug; 106:181-7.
Not within scope/ecological and fate	Givaudan N, Binet F, Le Bot B, Wiegand C	2014	Givaudan N, Binet F, Le Bot B, Wiegand C.Earthworm tolerance to residual agricultural pesticide contamination: field and experimental assessment of detoxification capabilities. Environ Pollut. 2014 Sep; 192:9-18.
In vitro/no glyphosate measurements	Roustan A, Aye M, De Meo M, Di Giorgio C	2014	Roustan A, Aye M, De Meo M, Di Giorgio C.Genotoxicity of mixtures of glyphosate and atrazine and their environmental transformation products before and after photoactivation. Chemosphere. 2014 Aug; 108:93-100.
Not within scope/ecological and fate	Huang Y, Reddy KN, Thomson SJ, Yao H	2015	Huang Y, Reddy KN, Thomson SJ, Yao H.Assessment of soybean injury from glyphosate using airborne multispectral remote sensing. Pest Manag Sci. 2015 Apr; 71(4):545-52.
Not within scope/ecological and fate	Baker LF, Mudge JF, Houlahan JE, Thompson DG, Kidd KA	2014	Baker LF, Mudge JF, Houlahan JE, Thompson DG, Kidd KA.The direct and indirect effects of a glyphosate-based herbicide and nutrients on Chironomidae (Diptera) emerging from small wetlands. Environ Toxicol Chem. 2014 Sep; 33(9):2076-85.
Not within scope/ecological and fate	GarcÃa-Torres T, GiuffrÃ© L, Romaniuk R, RÃos RP, Pagano EA	2014	GarcÃa-Torres T, GiuffrÃ© L, Romaniuk R, RÃos RP, Pagano EA.Exposure assessment to glyphosate of two species of annelids. Bull Environ Contam Toxicol. 2014 Aug; 93(2):209-14.
Poisoning or incident	Mahendrakar K, Venkategowda PM, Rao SM, Mutkule DP	2014	Mahendrakar K, Venkategowda PM, Rao SM, Mutkule DP.Glyphosate surfactant herbicide poisoning and management. Indian J Crit Care Med. 2014 May; 18(5):328-30.

Comments	Authors	PubDate (Year)	Full Citation
Not within scope/ecological and fate	Samanta P, Pal S, Mukherjee AK, Ghosh AR	2014	Samanta P, Pal S, Mukherjee AK, Ghosh AR.Biochemical effects of glyphosate based herbicide, Excel Mera 71 on enzyme activities of acetylcholinesterase (AChE), lipid peroxidation (LPO), catalase (CAT), glutathione-S-transferase (GST) and protein content on teleostean fishes. Ecotoxicol Environ Saf. 2014 Sep; 107:120-5.
In vitro/formulation/no glyphosate measurements	Cassault-Meyer E, Gress S, SÃ©ralini GÃ‰, Galeraud-Denis I	2014	Cassault-Meyer E, Gress S, SÃ©ralini GÃ‰, Galeraud-Denis I.An acute exposure to glyphosate-based herbicide alters aromatase levels in testis and sperm nuclear quality. Environ Toxicol Pharmacol. 2014 Jul; 38(1):131-40.
Not within scope/ecological and fate	Navarro CD, Martinez CB	2014	Navarro CD, Martinez CB.Effects of the surfactant polyoxyethylene amine (POEA) on genotoxic, biochemical and physiological parameters of the freshwater teleost Prochilodus lineatus. Comp Biochem Physiol C Toxicol Pharmacol. 2014 Sep; 165:83-90.
Poisoning or incident	Nishiyori Y, Nishida M, Shioda K, Suda S, Kato S	2014	Nishiyori Y, Nishida M, Shioda K, Suda S, Kato S.Unilateral hippocampal infarction associated with an attempted suicide: a case report. J Med Case Rep. 2014 Jun 23; 8:219.
Not within scope/ methods generating	Ewald M, Tetard L, Elie-Caille C, Nicod L, Passian A, Bourillot E, Lesniewska E	2014	Ewald M, Tetard L, Elie-Caille C, Nicod L, Passian A, Bourillot E, Lesniewska E.From surface to intracellular non-invasive nanoscale study of living cells impairments. Nanotechnology. 2014 Jul 25; 25(29):295101.
Enzyme activity/no gylphosate measurements/no histopath changes observed in liver, kidney, and small intestine	Larsen K, Najle R, Lifschitz A, MatÃ© ML, Lanusse C, Virkel GL	2014	Larsen K, Najle R, Lifschitz A, MatÃ© ML, Lanusse C, Virkel GL.Effects of Sublethal Exposure to a Glyphosate-Based Herbicide Formulation on Metabolic Activities of Different Xenobiotic-Metabolizing Enzymes in Rats. Int J Toxicol. 2014 Jul 1; 33(4):307-318.
In vitro/formulation/no glyphosate measurements	Coalova I, RÃos de Molina Mdel C, Chaufan G	2014	Coalova I, RÃos de Molina Mdel C, Chaufan G.Influence of the spray adjuvant on the toxicity effects of a glyphosate formulation. Toxicol In Vitro. 2014 Oct; 28(7):1306-11.

Comments	Authors	PubDate (Year)	Full Citation
Not within scope/ecological and fate	Maillard E, Imfeld G	2014	Maillard E, Imfeld G.Pesticide mass budget in a stormwater wetland. Environ Sci Technol. 2014 Aug 5; 48(15):8603-11.
Not within scope/ecological and fate	Zaller JG, Heigl F, Ruess L, Grabmaier A	2014	Zaller JG, Heigl F, Ruess L, Grabmaier A.Glyphosate herbicide affects belowground interactions between earthworms and symbiotic mycorrhizal fungi in a model ecosystem. Sci Rep. 2014 Jul 9; 4:5634.
Not within scope/ exposure and monitoring	Cao L, Deng T, Liang S, Tan X, Meng J	2014	Cao L, Deng T, Liang S, Tan X, Meng J.Determination of herbicides and its metabolite in soil and water samples by capillary electrophoresis-laser induced fluorescence detection using microwave-assisted derivatization. Anal Sci. 2014; 30(7):759-66.
Not within scope/ecological and fate	Kreutz LC, Pavan TR, Alves AG, Correia AG, Barriquel B, Santos ED, Barcellos LJ	2014	Kreutz LC, Pavan TR, Alves AG, Correia AG, Barriquel B, Santos ED, Barcellos LJ.Increased immunoglobulin production in silver catfish (Rhamdia quelen) exposed to agrichemicals. Braz J Med Biol Res. 2014 Jun; 47(6):499-504.
Not within scope/ecological and fate	Bai Y, Bao YB, Cai XL, Chen CH, Ye XC	2014	Bai Y, Bao YB, Cai XL, Chen CH, Ye XC.Feasibility of disposing waste glyphosate neutralization liquor with cement rotary kiln. J Hazard Mater. 2014 Aug 15; 278:500-5.
Not within scope/ exposure and monitoring	Ruiz-Toledo J, Castro R, Rivero-PÃ©rez N, Bello-Mendoza R, SÃ¡nchez D	2014	Ruiz-Toledo J, Castro R, Rivero-PÃ©rez N, Bello-Mendoza R, SÃ¡nchez D.Occurrence of glyphosate in water bodies derived from intensive agriculture in a tropical region of southern Mexico. Bull Environ Contam Toxicol. 2014 Sep; 93(3):289-93.
Not within scope/ecological and fate	Samanta P, Pal S, Mukherjee AK, Ghosh AR	2014	Samanta P, Pal S, Mukherjee AK, Ghosh AR.Evaluation of metabolic enzymes in response to Excel Mera 71, a glyphosate-based herbicide, and recovery pattern in freshwater teleostean fishes. Biomed Res Int. 2014; 2014:425159.
Not within scope/ hypothesis generating	Morley WA, Seneff S	2014	Morley WA, Seneff S.Diminished brain resilience syndrome: A modern day neurological pathology of increased susceptibility to mild brain trauma, concussion, and downstream neurodegeneration. Surg Neurol Int. 2014; 5:97.
Not within scope/ecological and fate	Zhou CF, Wang YJ, Sun RJ, Liu C, Fan GP, Qin WX, Li CC, Zhou DM	2014	Zhou CF, Wang YJ, Sun RJ, Liu C, Fan GP, Qin WX, Li CC, Zhou DM.Inhibition effect of glyphosate on the acute and subacute toxicity of cadmium to earthworm Eisenia fetida. Environ Toxicol Chem. 2014 Oct; 33(10):2351-7.

Comments	Authors	PubDate (Year)	Full Citation
Not within scope/ecological and fate	Koakoski G, Quevedo RM, Ferreira D, Oliveira TA, da Rosa JG, de Abreu MS, Gusso D, Marqueze A, Kreutz LC, Giacomini AC, Fagundes M, Barcellos LJ	2014	Koakoski G, Quevedo RM, Ferreira D, Oliveira TA, da Rosa JG, de Abreu MS, Gusso D, Marqueze A, Kreutz LC, Giacomini AC, Fagundes M, Barcellos LJ.Agrichemicals chronically inhibit the cortisol response to stress in fish. Chemosphere. 2014 Oct; 112:85-91.
Not within scope/ methods generating	Minami T, Liu Y, Akdeniz A, Koutnik P, Esipenko NA, Nishiyabu R, Kubo Y, Anzenbacher P Jr	2014	Minami T, Liu Y, Akdeniz A, Koutnik P, Esipenko NA, Nishiyabu R, Kubo Y, Anzenbacher P Jr.Intramolecular indicator displacement assay for anions: supramolecular sensor for glyphosate. J Am Chem Soc. 2014 Aug 13; 136(32):11396-401.
Review	Duke SO	2015	Duke SO.Perspectives on transgenic, herbicide-resistant crops in the United States almost 20 years after introduction. Pest Manag Sci. 2015 May; 71(5):652-7.
Not within scope/ecological and fate	Demetrio PM, Bonetto C, Ronco AE	2014	Demetrio PM, Bonetto C, Ronco AE.The effect of cypermethrin, chlorpyrifos, and glyphosate active ingredients and formulations on Daphnia magna (Straus). Bull Environ Contam Toxicol. 2014 Sep; 93(3):268-73.
Not within scope/ecological and fate	Guilherme S, Santos MA, GaivÃ£o I, Pacheco M	2014	Guilherme S, Santos MA, GaivÃ£o I, Pacheco M.Are DNA-damaging effects induced by herbicide formulations (RoundupÂ® and GarlonÂ®) in fish transient and reversible upon cessation of exposure? Aquat Toxicol. 2014 Oct; 155:213-21.
Not within scope/ecological and fate	Herbert LT, VÃ¡zquez DE, Arenas A, Farina WM	2014	Herbert LT, VÃ¡zquez DE, Arenas A, Farina WM.Effects of field-realistic doses of glyphosate on honeybee appetitive behaviour. J Exp Biol. 2014 Oct 1; 217(Pt 19):3457-64.
Not within scope/ methods generating	Sung IH, Lee YW, Chung DS	2014	Sung IH, Lee YW, Chung DS.Liquid extraction surface analysis in-line coupled with capillary electrophoresis for direct analysis of a solid surface sample. Anal Chim Acta. 2014 Aug 1; 838:45-50.
Not within scope/ecological and fate	Carranza CS, Barberis CL, Chiacchiera SM, Magnoli CE	2014	Carranza CS, Barberis CL, Chiacchiera SM, Magnoli CE.Influence of the pesticides glyphosate, chlorpyrifos and atrazine on growth parameters of nonochratoxigenic Aspergillus section Nigri strains isolated from agricultural soils. J Environ Sci Health B. 2014; 49(10):747-55.
Formulation/no glyphosate measurements	Tizhe EV, Ibrahim ND, Fatihu MY, Igbokwe IO, George BD, Ambali SF, Shallangwa JM	2014	Tizhe EV, Ibrahim ND, Fatihu MY, Igbokwe IO, George BD, Ambali SF, Shallangwa JM.Serum biochemical assessment of hepatic and renal functions of rats during oral exposure to glyphosate with zinc. Comp Clin Path. 2014; 23:1043-1050.

Comments	Authors	PubDate (Year)	Full Citation
Model/environmental fate	Geng C, Haudin CS, Zhang Y, Lashermes G, Houot S, Garnier P	2015	Geng C, Haudin CS, Zhang Y, Lashermes G, Houot S, Garnier P.Modeling the release of organic contaminants during compost decomposition in soil. Chemosphere. 2015 Jan; 119:423-31.
Microbiota/exposure unknown/urine samples taken	SchrÃ¶dl W, KrÃ¼ger S, Konstantinova-MÃ¼ller T, Shehata AA, Rulff R, KrÃ¼ger M	2014	SchrÃ¶dl W, KrÃ¼ger S, Konstantinova-MÃ¼ller T, Shehata AA, Rulff R, KrÃ¼ger M.Possible effects of glyphosate on Mucorales abundance in the rumen of dairy cows in Germany. Curr Microbiol. 2014 Dec; 69(6):817-23.
Not within scope/ methods generating	Ramirez CE, Bellmund S, Gardinali PR	2014	Ramirez CE, Bellmund S, Gardinali PR.A simple method for routine monitoring of glyphosate and its main metabolite in surface waters using lyophilization and LC-FLD+MS/MS. Case study: canals with influence on Biscayne National Park. Sci Total Environ. 2014 Oct 15; 496:389-401.
Not within scope/ecological and fate	Lopes FM, Varela Junior AS, Corcini CD, da Silva AC, Guazzelli VG, Tavares G, da Rosa CE	2014	Lopes FM, Varela Junior AS, Corcini CD, da Silva AC, Guazzelli VG, Tavares G, da Rosa CE.Effect of glyphosate on the sperm quality of zebrafish Danio rerio. Aquat Toxicol. 2014 Oct; 155:322-6.
Not within scope/ecological and fate	Marques A, Guilherme S, GaivÃ£o I, Santos MA, Pacheco M	2014	Marques A, Guilherme S, GaivÃ£o I, Santos MA, Pacheco M.Progression of DNA damage induced by a glyphosate-based herbicide in fish (Anguilla anguilla) upon exposure and post-exposure periods--insights into the mechanisms of genotoxicity and DNA repair. Comp Biochem Physiol C Toxicol Pharmacol. 2014 Nov; 166:126-33.
Poisoning or incident	Jyoti W, Thabah MM, Rajagopalan S, Hamide A	2014	Jyoti W, Thabah MM, Rajagopalan S, Hamide A.Esophageal perforation and death following glyphosate poisoning. J Postgrad Med. 2014 Jul-Sep; 60(3):346-7.
Not within scope/ exposure and monitoring	BÃ¡ez ME, Fuentes E, Espina MJ, Espinoza J	2014	BÃ¡ez ME, Fuentes E, Espina MJ, Espinoza J.Determination of glyphosate and aminomethylphosphonic acid in aqueous soil matrices: a critical analysis of the 9-fluorenylmethyl chloroformate derivatization reaction and application to adsorption studies. J Sep Sci. 2014 Nov; 37(21):3125-32.
Not within scope/ methods generating	Raina-Fulton R	2014	Raina-Fulton R.A review of methods for the analysis of orphan and difficult pesticides: glyphosate, glufosinate, quaternary ammonium and phenoxy acid herbicides, and dithiocarbamate and phthalimide fungicides. J AOAC Int. 2014 Jul-Aug; 97(4):965-77.

Comments	Authors	PubDate (Year)	Full Citation
Not within scope/ecological and fate	Sinhorin VD, Sinhorin AP, Teixeira JM, MilÃ©ski KM, Hansen PC, Moeller PR, Moreira PS, Baviera AM, Loro VL	2014	Sinhorin VD, Sinhorin AP, Teixeira JM, MilÃ©ski KM, Hansen PC, Moeller PR, Moreira PS, Baviera AM, Loro VL.Metabolic and behavior changes in surubim acutely exposed to a glyphosate-based herbicide. Arch Environ Contam Toxicol. 2014 Nov; 67(4):659-67.
Not within scope/ecological and fate	Xu H, Zhu X, Wang H, Li J, Dong L	2013	Xu H, Zhu X, Wang H, Li J, Dong L.Mechanism of resistance to fenoxaprop in Japanese foxtail (Alopecurus japonicus) from China. Pestic Biochem Physiol. 2013 Sep; 107(1):25-31.
Not within scope/ methods generating	Watanabe D, Ohta H, Yamamuro T	2014	Watanabe D, Ohta H, Yamamuro T.Solid-phase extraction of phosphorous-containing amino acid herbicides from biological specimens with a zirconia-coated silica cartridge. J Chromatogr B Analyt Technol Biomed Life Sci. 2014 Oct 15; 969:69-76.
Not within scope/ methods generating	Puzio K, Claude B, Amalric L, Berho C, Grellet E, Bayoudh S, NehmÃ© R, Morin P	2014	Puzio K, Claude B, Amalric L, Berho C, Grellet E, Bayoudh S, NehmÃ© R, Morin P.Molecularly imprinted polymer dedicated to the extraction of glyphosate in natural waters. J Chromatogr A. 2014 Sep 26; 1361:1-8.
Not within scope/comparing herbicide effectiveness	Cusati RC, Barbosa LC, Maltha CR, Demuner AJ, Oliveros-Bastidas A, Silva AA	2015	Cusati RC, Barbosa LC, Maltha CR, Demuner AJ, Oliveros-Bastidas A, Silva AA.Tetraoxanes as a new class of efficient herbicides comparable with commercial products. Pest Manag Sci. 2015 Jul; 71(7):1037-48.
Not within scope/ecological and fate	Singh B, Singh K	2014	Singh B, Singh K.Microbial degradation of herbicides. Crit Rev Microbiol. 2014 Aug 27; :1-17.
Not within scope/ecological and fate	Kongsong P, Sikong L, Niyomwas S, Rachpech V	2014	Kongsong P, Sikong L, Niyomwas S, Rachpech V.Photocatalytic degradation of glyphosate in water by N-doped SnO2/TiO2 thin-film-coated glass fibers. Photochem Photobiol. 2014 Nov-Dec; 90(6):1243-50.

Comments	Authors	PubDate (Year)	Full Citation
Glyphosate measurements in air by ELISA/no glyphosate measurement for direct exposure/treatment directly to nose in suspension	Kumar S, Khodoun M, Kettleson EM, McKnight C, Reponen T, Grinshpun SA, Adhikari A	2014	Kumar S, Khodoun M, Kettleson EM, McKnight C, Reponen T, Grinshpun SA, Adhikari A.Glyphosate-rich air samples induce IL-33, TSLP and generate IL-13 dependent airway inflammation. Toxicology. 2014 Nov 5; 325:42-51.
Review/trend comparison	Nevison CD	2014	Nevison CD.A comparison of temporal trends in United States autism prevalence to trends in suspected environmental factors. Environ Health. 2014 Sep 5; 13:73.
Not within scope/ecological and fate	Nordborg M, Cederberg C, Berndes G	2014	Nordborg M, Cederberg C, Berndes G.Modeling potential freshwater ecotoxicity impacts due to pesticide use in biofuel feedstock production: the cases of maize, rapeseed, salix, soybean, sugar cane, and wheat. Environ Sci Technol. 2014 Oct 7; 48(19):11379-88.
Not within scope/ecological and fate	Forlani G, Bertazzini M, Barillaro D, Rippka R	2015	Forlani G, Bertazzini M, Barillaro D, Rippka R.Divergent properties and phylogeny of cyanobacterial 5-enol-pyruvyl-shikimate-3-phosphate synthases: evidence for horizontal gene transfer in the Nostocales. New Phytol. 2015 Jan; 205(1):160-71.
Not within scope/ methods generating	Liu Y, Bonizzoni M	2014	Liu Y, Bonizzoni M.A supramolecular sensing array for qualitative and quantitative analysis of organophosphates in water. J Am Chem Soc. 2014 Oct 8; 136(40):14223-9.
Poisoning or incident	Cherukuri H, Pramoda K, Rohini D, Thunga G, Vijaynarayana K, Sreedharan N, Varma M, Pandit V	2014	Cherukuri H, Pramoda K, Rohini D, Thunga G, Vijaynarayana K, Sreedharan N, Varma M, Pandit V.Demographics, clinical characteristics and management of herbicide poisoning in tertiary care hospital. Toxicol Int. 2014 May; 21(2):209-13.
Formulation/no glyphosate measurements	Tizhe EV, Ibrahim ND, Fatihu MY, Onyebuchi II, George BD, Ambali SF, Shallangwa JM	2014	Tizhe EV, Ibrahim ND, Fatihu MY, Onyebuchi II, George BD, Ambali SF, Shallangwa JM.Influence of zinc supplementation on histopathological changes in the stomach, liver, kidney, brain, pancreas and spleen during subchronic exposure of Wistar rats to glyphosate. Comp Clin Path. 2014; 23(5):1535-1543.

Comments	Authors	PubDate (Year)	Full Citation
Not within scope/ecological and fate	Sabatier P, Poulenard J, Fanget B, Reyss JL, Develle AL, Wilhelm B, Ployon E, Pignol C, Naffrechoux E, Dorioz JM, Montuelle B, Arnaud F	2014	Sabatier P, Poulenard J, Fanget B, Reyss JL, Develle AL, Wilhelm B, Ployon E, Pignol C, Naffrechoux E, Dorioz JM, Montuelle B, Arnaud F.Long-term relationships among pesticide applications, mobility, and soil erosion in a vineyard watershed. Proc Natl Acad Sci U S A. 2014 Nov 4; 111(44):15647-52.
Not within scope/pest management and weed resistance	Owen MD, Beckie HJ, Leeson JY, Norsworthy JK, Steckel LE	2015	Owen MD, Beckie HJ, Leeson JY, Norsworthy JK, Steckel LE.Integrated pest management and weed management in the United States and Canada. Pest Manag Sci. 2015 Mar; 71(3):357-76.
Review	Shaw CA, Seneff S, Kette SD, Tomljenovic L, Oller JW Jr, Davidson RM	2014	Shaw CA, Seneff S, Kette SD, Tomljenovic L, Oller JW Jr, Davidson RM.Aluminum-induced entropy in biological systems: implications for neurological disease. J Toxicol. 2014; 2014:491316.
No glyphosate measurements	Abarikwu SO, Akiri OF, Durojaiye MA, Adenike A	2015	Abarikwu SO, Akiri OF, Durojaiye MA, Adenike A.Combined effects of repeated administration of Bretmont Wipeout (glyphosate) and Ultrazin (atrazine) on testosterone, oxidative stress and sperm quality of Wistar rats. Toxicol Mech Methods. 2015 Jan; 25(1):70-80.
Not relevant	Seiber JN, Coats J, Duke SO, Gross AD	2014	Seiber JN, Coats J, Duke SO, Gross AD.Biopesticides: state of the art and future opportunities. J Agric Food Chem. 2014 Dec 3; 62(48):11613-9.
Microbiota/no glyphosate measurements	Ackermann W, Coenen M, SchrÃ¶dl W, Shehata AA, KrÃ¼ger M	2015	Ackermann W, Coenen M, SchrÃ¶dl W, Shehata AA, KrÃ¼ger M.The influence of glyphosate on the microbiota and production of botulinum neurotoxin during ruminal fermentation. Curr Microbiol. 2015 Mar; 70(3):374-82.
Not within scope/ecological and fate	Vidal E, Negro A, Cassano A, Zalazar C	2015	Vidal E, Negro A, Cassano A, Zalazar C.Simplified reaction kinetics, models and experiments for glyphosate degradation in water by the UV/H2O2 process. Photochem Photobiol Sci. 2015 Feb; 14(2):366-77.
Not within scope/ exposure and monitoring	Lanaro R, Costa JL, Cazenave SO, Zanolli-Filho LA, Tavares MF, Chasin AA	2015	Lanaro R, Costa JL, Cazenave SO, Zanolli-Filho LA, Tavares MF, Chasin AA.Determination of herbicides paraquat, glyphosate, and aminomethylphosphonic acid in marijuana samples by capillary electrophoresis. J Forensic Sci. 2015 Jan; 60 Suppl 1:S241-7.
In vitro/formulation/no glyphosate measurements	Gress S, Lemoine S, Puddu PE, SÃ©ralini GE, Rouet R	2015	Gress S, Lemoine S, Puddu PE, SÃ©ralini GE, Rouet R.Cardiotoxic Electrophysiological Effects of the Herbicide Roundup(Â®) in Rat and Rabbit Ventricular Myocardium In Vitro. Cardiovasc Toxicol. 2015 Oct; 15(4):324-35.

Comments	Authors	PubDate (Year)	Full Citation
Not within scope/ecological and fate	Topal A, Atamanalp M, UÃ§ar A, OruÃ§ E, Kocaman EM, Sulukan E, Akdemir F, Beydemir Åž, KÄ±lÄ±nÃ§ N, ErdoÄŸan O, Ceyhun SB	2015	Topal A, Atamanalp M, UÃ§ar A, OruÃ§ E, Kocaman EM, Sulukan E, Akdemir F, Beydemir Åž, KÄ±lÄ±nÃ§ N, ErdoÄŸan O, Ceyhun SB.Effects of glyphosate on juvenile rainbow trout (Oncorhynchus mykiss): transcriptional and enzymatic analyses of antioxidant defence system, histopathological liver damage and swimming performance. Ecotoxicol Environ Saf. 2015 Jan; 111:206-14.
Not within scope/ecological and fate	Mottier A, SÃ©guin A, Devos A, Pabic CL, Voiseux C, Lebel JM, Serpentini A, Fievet B, Costil K	2015	Mottier A, SÃ©guin A, Devos A, Pabic CL, Voiseux C, Lebel JM, Serpentini A, Fievet B, Costil K.Effects of subchronic exposure to glyphosate in juvenile oysters (Crassostrea gigas): From molecular to individual levels. Mar Pollut Bull. 2015 Jun 30; 95(2):665-77.
Not within scope/ecological and fate	Castro Ade J, Colares IG, Franco TC, Cutrim MV, Luvizotto-Santos R	2015	Castro Ade J, Colares IG, Franco TC, Cutrim MV, Luvizotto-Santos R.Using a toxicity test with Ruppia maritima (Linnaeus) to assess the effects of Roundup. Mar Pollut Bull. 2015 Feb 28; 91(2):506-10.
Model/environmental fate	Mariani ML, Romero RL, Zalazar CS	2015	Mariani ML, Romero RL, Zalazar CS.Modeling of degradation kinetic and toxicity evaluation of herbicides mixtures in water using the UV/H2O2 process. Photochem Photobiol Sci. 2015 Mar; 14(3):608-17.
Not within scope/pest management and weed resistance	Sells SM, Held DW, Enloe SF, Loewenstein NJ, Eckhardt LG	2015	Sells SM, Held DW, Enloe SF, Loewenstein NJ, Eckhardt LG.Impact of cogongrass management strategies on generalist predators in cogongrass-infested longleaf pine plantations. Pest Manag Sci. 2015 Mar; 71(3):478-84.
Not within scope/ecological and fate	Lima IS, Baumeier NC, Rosa RT, Campelo PM, Rosa EA	2014	Lima IS, Baumeier NC, Rosa RT, Campelo PM, Rosa EA.Influence of glyphosate in planktonic and biofilm growth of Pseudomonas aeruginosa. Braz J Microbiol. 2014; 45(3):971-5.
Not relevant	Pope MA, Spence E, Seralvo V, Gacesa R, Heidelberger S, Weston AJ, Dunlap WC, Shick JM, Long PF	2015	Pope MA, Spence E, Seralvo V, Gacesa R, Heidelberger S, Weston AJ, Dunlap WC, Shick JM, Long PF.O-Methyltransferase is shared between the pentose phosphate and shikimate pathways and is essential for mycosporine-like amino acid biosynthesis in Anabaena variabilis ATCC 29413. Chembiochem. 2015 Jan 19; 16(2):320-7.
Not within scope/ecological and fate	Severns PM, Estep LK, Sackett KE, Mundt CC	2014	Severns PM, Estep LK, Sackett KE, Mundt CC.Degree of host susceptibility in the initial disease outbreak influences subsequent epidemic spread. J Appl Ecol. 2014 Dec 1; 51(6):1622-1630.

Comments	Authors	PubDate (Year)	Full Citation
Not within scope/ecological and fate	Zhu X, Li B, Yang J, Li Y, Zhao W, Shi J, Gu J	2015	Zhu X, Li B, Yang J, Li Y, Zhao W, Shi J, Gu J.Effective adsorption and enhanced removal of organophosphorus pesticides from aqueous solution by Zr-based MOFs of UiO-67. ACS Appl Mater Interfaces. 2015 Jan 14; 7(1):223-31.
Not within scope/ecological and fate	Marques A, Guilherme S, GaivÃ£o I, Santos MA, Pacheco M	2014	Marques A, Guilherme S, GaivÃ£o I, Santos MA, Pacheco M.Erratum to: Progression of DNA damage induced by a glyphosate-based herbicide in fish (Anguilla anguilla) upon exposure and post-exposure periods - Insights into the mechanisms of genotoxicity and DNA repair' [Comp. Biochem. Physiol. C 166 (2014) 126-133]. Comp Biochem Physiol C Toxicol Pharmacol. 2014 Nov 8; 168C:1.
Dose injected/no glyphosate measurements/	HernÃ¡ndez-Plata I, Giordano M, DÃaz-MuÃ±oz M, RodrÃguez VM	2015	HernÃ¡ndez-Plata I, Giordano M, DÃaz-MuÃ±oz M, RodrÃguez VM.The herbicide glyphosate causes behavioral changes and alterations in dopaminergic markers in male Sprague-Dawley rat. Neurotoxicology. 2015 Jan; 46:79-91.
Not within scope/pest management and weed resistance	Saraiva Ade S, Sarmento RA, Erasmo EA, Pedro-Neto M, de Souza DJ, Teodoro AV, Silva DG	2015	Saraiva Ade S, Sarmento RA, Erasmo EA, Pedro-Neto M, de Souza DJ, Teodoro AV, Silva DG.Weed management practices affect the diversity and relative abundance of physic nut mites. Exp Appl Acarol. 2015 Mar; 65(3):359-75.
Not within scope/ecological and fate	BÃ¡ez ME, Espinoza J, Silva R, Fuentes E	2015	BÃ¡ez ME, Espinoza J, Silva R, Fuentes E.Sorption-desorption behavior of pesticides and their degradation products in volcanic and nonvolcanic soils: interpretation of interactions through two-way principal component analysis. Environ Sci Pollut Res Int. 2015 Jun; 22(11):8576-85.
Not within scope/ecological and fate	Gaines TA, Ward SM, Bukun B, Preston C, Leach JE, Westra P	2012	Gaines TA, Ward SM, Bukun B, Preston C, Leach JE, Westra P.Interspecific hybridization transfers a previously unknown glyphosate resistance mechanism in Amaranthus species. Evol Appl. 2012 Jan; 5(1):29-38.
Poisoning or incident	You MJ, Shin GW, Lee CS	2015	You MJ, Shin GW, Lee CS.Clostridium tertium bacteremia in a patient with glyphosate ingestion. Am J Case Rep. 2015 Jan 6; 16:4-7.
Review	He S, Tang G	2014	He S, Tang G.[The review of study on glyphosate hebicide toxic effects]. Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi. 2014 Nov; 32(11):868-71.
Not within scope/ methods generating	Carneiro RT, Taketa TB, Gomes Neto RJ, Oliveira JL, Campos EV, de Moraes MA,	2015	Carneiro RT, Taketa TB, Gomes Neto RJ, Oliveira JL, Campos EV, de Moraes MA, da Silva CM, Beppu MM, Fraceto LF.Removal of glyphosate herbicide from water using biopolymer membranes. J Environ Manage. 2015 Mar 15; 151:353-60.

Comments	Authors	PubDate (Year)	Full Citation
	da Silva CM, Beppu MM, Fraceto LF		
Epidemiology	Jayasumana C, Paranagama P, Agampodi S, Wijewardane C, Gunatilake S, Siribaddana S	2015	Jayasumana C, Paranagama P, Agampodi S, Wijewardane C, Gunatilake S, Siribaddana S.Drinking well water and occupational exposure to Herbicides is associated with chronic kidney disease, in Padavi-Sripura, Sri Lanka. Environ Health. 2015 Jan 18; 14:6.
Not within scope/ecological and fate	Kanissery RG, Welsh A, Sims GK	2015	Kanissery RG, Welsh A, Sims GK.Effect of soil aeration and phosphate addition on the microbial bioavailability of carbon-14-glyphosate. J Environ Qual. 2015 Jan; 44(1):137-44.
Not within scope/ecological and fate	Rampoldi EA, Hang S, Barriuso E	2014	Rampoldi EA, Hang S, Barriuso E.Carbon-14-glyphosate behavior in relationship to pedoclimatic conditions and crop sequence. J Environ Qual. 2014 Mar; 43(2):558-67.
Not within scope/ methods generating	An K, Gao L	2014	An K, Gao L.[Study on the method for determination of glyphosate in workplace air by HPLC post-column derivatization]. Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi. 2014 Dec; 32(12):934-5.
Not within scope/ecological and fate	Ribeiro DN, Nandula VK, Dayan FE, Rimando AM, Duke SO, Reddy KN, Shaw DR	2015	Ribeiro DN, Nandula VK, Dayan FE, Rimando AM, Duke SO, Reddy KN, Shaw DR.Possible glyphosate tolerance mechanism in pitted morningglory (Ipomoea lacunosa L.). J Agric Food Chem. 2015 Feb 18; 63(6):1689-97.
Not within scope/ecological and fate	Peixoto MM, Bauerfeldt GF, Herbst MH, Pereira MS, da Silva CO	2015	Peixoto MM, Bauerfeldt GF, Herbst MH, Pereira MS, da Silva CO.Study of the stepwise deprotonation reactions of glyphosate and the corresponding pKa values in aqueous solution. J Phys Chem A. 2015 May 28; 119(21):5241-9.
Epidemiology	Sorahan T	2015	Sorahan T.Multiple myeloma and glyphosate use: a re-analysis of US Agricultural Health Study (AHS) data. Int J Environ Res Public Health. 2015 Jan 28; 12(2):1548-59.
Not within scope/ecological and fate	Uren Webster TM, Santos EM	2015	Uren Webster TM, Santos EM.Global transcriptomic profiling demonstrates induction of oxidative stress and of compensatory cellular stress responses in brown trout exposed to glyphosate and Roundup. BMC Genomics. 2015 Jan 31; 16:32.

Comments	Authors	PubDate (Year)	Full Citation
Not within scope/ecological and fate	MenÃ©ndez-Helman RJ, Miranda LA, Dos Santos Afonso M, SalibiÃ¡n A	2015	MenÃ©ndez-Helman RJ, Miranda LA, Dos Santos Afonso M, SalibiÃ¡n A.Subcellular energy balance of Odontesthes bonariensis exposed to a glyphosate-based herbicide. Ecotoxicol Environ Saf. 2015 Apr; 114:157-63.
Not within scope/ecological and fate	Chen X, Liu F, Liu B, Tian L, Hu W, Xia Q	2015	Chen X, Liu F, Liu B, Tian L, Hu W, Xia Q.A novel route to graphite-like carbon supporting SnO2 with high electron transfer and photocatalytic activity. J Hazard Mater. 2015 Apr 28; 287:126-32.
Not within scope/ecological and fate	Yang X, Wang F, Bento CP, Xue S, Gai L, van Dam R, Mol H, Ritsema CJ, Geissen V	2015	Yang X, Wang F, Bento CP, Xue S, Gai L, van Dam R, Mol H, Ritsema CJ, Geissen V.Short-term transport of glyphosate with erosion in Chinese loess soil--a flume experiment. Sci Total Environ. 2015 Apr 15; 512-513:406-14.
Not within scope/ecological and fate	Sura S, Waiser MJ, Tumber V, Raina-Fulton R, Cessna AJ	2015	Sura S, Waiser MJ, Tumber V, Raina-Fulton R, Cessna AJ.Effects of a herbicide mixture on primary and bacterial productivity in four prairie wetlands with varying salinities: an enclosure approach. Sci Total Environ. 2015 Apr 15; 512-513:526-39.
Not within scope/ecological and fate	Marble SC, Prior SA, Runion GB, Torbert HA	2015	Marble SC, Prior SA, Runion GB, Torbert HA.Control of yellow and purple nutsedge in elevated CO2 environments with glyphosate and halosulfuron. Front Plant Sci. 2015; 6:1.
Epidemiology	Cortinovis C, Davanzo F, Rivolta M, Caloni F	2015	Cortinovis C, Davanzo F, Rivolta M, Caloni F.Glyphosate-surfactant herbicide poisoning in domestic animals: an epidemiological survey. Vet Rec. 2015 Apr 18; 176(16):413.
Not within scope/ methods generating	Wei X, Pu Q	2015	Wei X, Pu Q.Microchip electrophoresis for fast and interference-free determination of trace amounts of glyphosate and glufosinate residues in agricultural products. Methods Mol Biol. 2015; 1274:21-9.
Not within scope/ecological and fate	Douros DL, Gaines KF, Novak JM	2015	Douros DL, Gaines KF, Novak JM.Atrazine and glyphosate dynamics in a lotic ecosystem: the common snapping turtle as a sentinel species. Environ Monit Assess. 2015 Mar; 187(3):114.
Review	Kier LD	2015	Kier LD.Review of genotoxicity biomonitoring studies of glyphosate-based formulations. Crit Rev Toxicol. 2015 Mar; 45(3):209-18.
Not within scope/ecological and fate	Levine SL, von MÃ©rey G, Minderhout T, Manson P, Sutton P	2015	Levine SL, von MÃ©rey G, Minderhout T, Manson P, Sutton P.Aminomethylphosphonic acid has low chronic toxicity to Daphnia magna and Pimephales promelas. Environ Toxicol Chem. 2015 Jun; 34(6):1382-9.

Comments	Authors	PubDate (Year)	Full Citation
Not within scope/ exposure and monitoring	Tang T, BoÃ«nne W, Desmet N, Seuntjens P, Bronders J, van Griensven A	2015	Tang T, BoÃ«nne W, Desmet N, Seuntjens P, Bronders J, van Griensven A.Quantification and characterization of glyphosate use and loss in a residential area. Sci Total Environ. 2015 Jun 1; 517:207-14.
Not within scope/ecological and fate	Ma J, Li X	2015	Ma J, Li X.Alteration in the cytokine levels and histopathological damage in common carp induced by glyphosate. Chemosphere. 2015 Jun; 128:293-8.
Not within scope/ exposure and monitoring	Jayasumana C, Fonseka S, Fernando A, Jayalath K, Amarasinghe M, Siribaddana S, Gunatilake S, Paranagama P	2015	Jayasumana C, Fonseka S, Fernando A, Jayalath K, Amarasinghe M, Siribaddana S, Gunatilake S, Paranagama P.Phosphate fertilizer is a main source of arsenic in areas affected with chronic kidney disease of unknown etiology in Sri Lanka. Springerplus. 2015; 4:90.
Not within scope/crop composition	Curran KL, Festa AR, Goddard SD, Harrigan GG, Taylor ML	2015	Curran KL, Festa AR, Goddard SD, Harrigan GG, Taylor ML.Kernel compositions of glyphosate-tolerant and corn rootworm-protected MON 88017 sweet corn and insect-protected MON 89034 sweet corn are equivalent to that of conventional sweet corn (Zea mays). J Agric Food Chem. 2015 Mar 25; 63(11):3046-52.
Not within scope/ecological and fate	Sasal MC, Demonte L, Cislaghi A, Gabioud EA, Oszust JD, Wilson MG, Michlig N, BeldomÃ©nico HR, Repetti MR	2015	Sasal MC, Demonte L, Cislaghi A, Gabioud EA, Oszust JD, Wilson MG, Michlig N, BeldomÃ©nico HR, Repetti MR.Glyphosate loss by runoff and its relationship with phosphorus fertilization. J Agric Food Chem. 2015 May 13; 63(18):4444-8.
Not within scope/ecological and fate	Levis NA, Johnson JR	2015	Levis NA, Johnson JR.Level of UV-B radiation influences the effects of glyphosate-based herbicide on the spotted salamander. Ecotoxicology. 2015 Jul; 24(5):1073-86.
News article	Guyton KZ, Loomis D, Grosse Y, El Ghissassi F, Benbrahim-Tallaa L, Guha N, Scoccianti C, Mattock H, Straif K	2015	Guyton KZ, Loomis D, Grosse Y, El Ghissassi F, Benbrahim-Tallaa L, Guha N, Scoccianti C, Mattock H, Straif K.Carcinogenicity of tetrachlorvinphos, parathion, malathion, diazinon, and glyphosate. Lancet Oncol. 2015 May; 16(5):490-1.
Effect on E. coli and Salmonella/formulation	Kurenbach B, Marjoshi D, AmÃ¡bile-Cuevas CF, Ferguson GC, Godsoe W, Gibson P, Heinemann JA	2015	Kurenbach B, Marjoshi D, AmÃ¡bile-Cuevas CF, Ferguson GC, Godsoe W, Gibson P, Heinemann JA.Sublethal exposure to commercial formulations of the herbicides dicamba, 2,4-dichlorophenoxyacetic acid, and glyphosate cause changes in antibiotic susceptibility in Escherichia coli and Salmonella enterica serovar Typhimurium. MBio. 2015 Mar 24; 6(2)

Comments	Authors	PubDate (Year)	Full Citation
Not within scope/pest management and weed resistance	Evans JA, Tranel PJ, Hager AG, Schutte B, Wu C, Chatham LA, Davis AS	2015	Evans JA, Tranel PJ, Hager AG, Schutte B, Wu C, Chatham LA, Davis AS.Managing the evolution of herbicide resistance. Pest Manag Sci. 2015 Mar 24;
Review	Khamitova RY, Mirsaitova GT	2014	Khamitova RY, Mirsaitova GT.[Current trends in the use of pesticides]. Gig Sanit. 2014 Jul-Aug; (4):23-6.
In vitro/no glyphosate measurements/glyphosate used as neg. control	Alloisio S, Nobile M, Novellino A	2015	Alloisio S, Nobile M, Novellino A.Multiparametric characterisation of neuronal network activity for in vitro agrochemical neurotoxicity assessment. Neurotoxicology. 2015 May; 48:152-65.
Not within scope/ methods generating	Meyer-Monath M, Chatellier C, Cabooter D, Rouget F, Morel I, Lestremau F	2015	Meyer-Monath M, Chatellier C, Cabooter D, Rouget F, Morel I, Lestremau F.Development of liquid chromatography methods coupled to mass spectrometry for the analysis of substances with a wide variety of polarity in meconium. Talanta. 2015 Jun 1; 138:231-9.
Not within scope/ecological and fate	Ki SJ, Ray C, Hantush MM	2015	Ki SJ, Ray C, Hantush MM.Applying a statewide geospatial leaching tool for assessing soil vulnerability ratings for agrochemicals across the contiguous United States. Water Res. 2015 Jun 15; 77:107-18.
Not within scope/ecological and fate	Braz-Mota S, Sadauskas-Henrique H, Duarte RM, Val AL, Almeida-Val VM	2015	Braz-Mota S, Sadauskas-Henrique H, Duarte RM, Val AL, Almeida-Val VM.RoundupÂ® exposure promotes gills and liver impairments, DNA damage and inhibition of brain cholinergic activity in the Amazon teleost fish Colossoma macropomum. Chemosphere. 2015 Sep; 135:53-60.
Not within scope/crop composition	Harrigan GG, Skogerson K, MacIsaac S, Bickel A, Perez T, Li X	2015	Harrigan GG, Skogerson K, MacIsaac S, Bickel A, Perez T, Li X.Application of (1)h NMR profiling to assess seed metabolomic diversity. A case study on a soybean era population. J Agric Food Chem. 2015 May 13; 63(18):4690-7.
Poisoning or incident	Thakur DS, Khot R, Joshi PP, Pandharipande M, Nagpure K	2014	Thakur DS, Khot R, Joshi PP, Pandharipande M, Nagpure K.Glyphosate poisoning with acute pulmonary edema. Toxicol Int. 2014 Sep-Dec; 21(3):328-30.
Not within scope/ecological and fate	Zabaloy MC, CarnÃ© I, Viassolo R, GÃ³mez MA, Gomez E	2015	Zabaloy MC, CarnÃ© I, Viassolo R, GÃ³mez MA, Gomez E.Soil ecotoxicity assessment of glyphosate use under field conditions: microbial activity and community structure of Eubacteria and ammonia-oxidising bacteria. Pest Manag Sci. 2015 May 9;

Comments	Authors	PubDate (Year)	Full Citation
Not within scope/ methods generating	Mogusu EO, Wolbert JB, Kujawinski DM, Jochmann MA, Elsner M	2015	Mogusu EO, Wolbert JB, Kujawinski DM, Jochmann MA, Elsner M.Dual element ((15)N/(14)N, (13)C/(12)C) isotope analysis of glyphosate and AMPA by derivatization-gas chromatography isotope ratio mass spectrometry (GC/IRMS) combined with LC/IRMS. Anal Bioanal Chem. 2015 Jul; 407(18):5249-60.
Not within scope/pest management and weed resistance	Gibson DJ, Young BG, Owen MD, Gage KL, Matthews JL, Jordan DL, Shaw DR, Weller SC, Wilson RG	2015	Gibson DJ, Young BG, Owen MD, Gage KL, Matthews JL, Jordan DL, Shaw DR, Weller SC, Wilson RG.Benchmark study on glyphosate-resistant cropping systems in the United States. Part 7: Effects of weed management strategy (grower practices versus academic recommendations) on the weed soil seedbank over 6 years. Pest Manag Sci. 2015 May 14;
Not within scope/ methods generating	Fauvelle V, Nhu-Trang TT, Feret T, Madarassou K, Randon J, Mazzella N	2015	Fauvelle V, Nhu-Trang TT, Feret T, Madarassou K, Randon J, Mazzella N.Evaluation of Titanium Dioxide as a Binding Phase for the Passive Sampling of Glyphosate and Aminomethyl Phosphonic Acid in an Aquatic Environment. Anal Chem. 2015 Jun 16; 87(12):6004-9.
Not within scope/ exposure and monitoring	Liu G, Peng Z, Lan T, Xu X, Huang G, Yu S, Liu G, Li J	2015	Liu G, Peng Z, Lan T, Xu X, Huang G, Yu S, Liu G, Li J.[Health risk assessment on pesticide residues in drinking water in Shenzhen]. Wei Sheng Yan Jiu. 2015 Mar; 44(2):264-9.
Treatment/AMPA	Parajuli KR, Zhang Q, Liu S, You Z	2015	Parajuli KR, Zhang Q, Liu S, You Z.Aminomethylphosphonic acid and methoxyacetic acid induce apoptosis in prostate cancer cells. Int J Mol Sci. 2015 May 22; 16(5):11750-65.
In vitro/no glyphosate measurements	Yao P, Lin Y, Wu G, Lu Y, Zhan T, Kumar A, Zhang L, Liu Z	2015	Yao P, Lin Y, Wu G, Lu Y, Zhan T, Kumar A, Zhang L, Liu Z.Improvement of glycine oxidase by DNA shuffling, and site-saturation mutagenesis of F247 residue. Int J Biol Macromol. 2015 Aug; 79:965-70.
Not within scope/ecological and fate	Yang X, Wang F, Bento CP, Meng L, van Dam R, Mol H, Liu G, Ritsema CJ, Geissen V	2015	Yang X, Wang F, Bento CP, Meng L, van Dam R, Mol H, Liu G, Ritsema CJ, Geissen V.Decay characteristics and erosion-related transport of glyphosate in Chinese loess soil under field conditions. Sci Total Environ. 2015 Oct 15; 530-531:87-95.
In vitro/no glyphosate measurements	Wallace K, Strickland JD, Valdivia P, Mundy WR, Shafer TJ	2015	Wallace K, Strickland JD, Valdivia P, Mundy WR, Shafer TJ.A multiplexed assay for determination of neurotoxicant effects on spontaneous network activity and viability from microelectrode arrays. Neurotoxicology. 2015 Jul; 49:79-85.

Comments	Authors	PubDate (Year)	Full Citation
Not within scope/ecological and fate	Zhang K, Deletic A, Page D, McCarthy DT	2015	Zhang K, Deletic A, Page D, McCarthy DT.Surrogates for herbicide removal in stormwater biofilters. Water Res. 2015 Sep 15; 81:64-71.
Not within scope/ecological and fate	SvobodovÃ¡ Z, SkokovÃ¡ HabuÅ¡tovÃ¡ O, Hutchison WD, Hussein HM, Sehnal F	2015	SvobodovÃ¡ Z, SkokovÃ¡ HabuÅ¡tovÃ¡ O, Hutchison WD, Hussein HM, Sehnal F.Risk Assessment of Genetically Engineered Maize Resistant to Diabrotica spp.: Influence on Above-Ground Arthropods in the Czech Republic. PLoS One. 2015; 10(6):e0130656.
Not within scope/ exposure and monitoring	Mesnage R, Defarge N, Rocque LM, Spiroux de VendÃ´mois J, SÃ©ralini GE	2015	Mesnage R, Defarge N, Rocque LM, Spiroux de VendÃ´mois J, SÃ©ralini GE.Laboratory Rodent Diets Contain Toxic Levels of Environmental Contaminants: Implications for Regulatory Tests. PLoS One. 2015; 10(7):e0128429.
Not within scope/ecological and fate	Rocha TL, Santos AP, Yamada Ã T, Soares CM, Borges CL, BailÃ£o AM, SabÃ³ia-Morais SM	2015	Rocha TL, Santos AP, Yamada Ã T, Soares CM, Borges CL, BailÃ£o AM, SabÃ³ia-Morais SM.Proteomic and histopathological response in the gills of Poecilia reticulata exposed to glyphosate-based herbicide. Environ Toxicol Pharmacol. 2015 Jul; 40(1):175-86.
Not within scope/ exposure and monitoring	Luo X, Chen L, Zhao Y	2015	Luo X, Chen L, Zhao Y.Simultaneous determination of three chloroacetic acids, three herbicides, and 12 anions in water by ion chromatography. J Sep Sci. 2015 Sep; 38(17):3096-102.
Not within scope/ecological and fate	Allegrini M, Zabaloy MC, GÃ³mez Edel V	2015	Allegrini M, Zabaloy MC, GÃ³mez Edel V.Ecotoxicological assessment of soil microbial community tolerance to glyphosate. Sci Total Environ. 2015 Nov 15; 533:60-8.
Not within scope/ecological and fate	Baurand PE, Capelli N, Vaufleury Ad	2015	Baurand PE, Capelli N, Vaufleury Ad.Genotoxicity assessment of pesticides on terrestrial snail embryos by analysis of random amplified polymorphic DNA profiles. J Hazard Mater. 2015 Nov 15; 298:320-7.
Not within scope/ exposure and monitoring	Jayasumana C, Gunatilake S, Siribaddana S	2015	Jayasumana C, Gunatilake S, Siribaddana S.Simultaneous exposure to multiple heavy metals and glyphosate may contribute to Sri Lankan agricultural nephropathy. BMC Nephrol. 2015 Jul 11; 16:103.
Not within scope/ecological and fate	Sol Balbuena M, Tison L, Hahn ML, Greggers U, Menzel R, Farina WM	2015	Sol Balbuena M, Tison L, Hahn ML, Greggers U, Menzel R, Farina WM.Effects of sub-lethal doses of glyphosate on honeybee navigation. J Exp Biol. 2015 Jul 10;

Comments	Authors	PubDate (Year)	Full Citation
Not within scope/ecological and fate	Salem FB, Said OB, Aissa P, Mahmoudi E, Monperrus M, Grunberger O, Duran R	2015	Salem FB, Said OB, Aissa P, Mahmoudi E, Monperrus M, Grunberger O, Duran R.Pesticides in Ichkeul Lake-Bizerta Lagoon Watershed in Tunisia: use, occurrence, and effects on bacteria and free-living marine nematodes. Environ Sci Pollut Res Int. 2015 Jul 14;
Not within scope/ecological and fate	Horak MJ, Rosenbaum EW, Phillips SL, Kendrick DL, Carson D, Clark PL, Nickson TE	2015	Horak MJ, Rosenbaum EW, Phillips SL, Kendrick DL, Carson D, Clark PL, Nickson TE.Characterization of the ecological interactions of Roundup Ready 2 YieldÂ® soybean, MON 89788, for use in ecological risk assessment. GM Crops Food. 2015 Jul 3; 6(3):167-82.
Not within scope/ecological and fate	Struger J, Van Stempvoort DR, Brown SJ	2015	Struger J, Van Stempvoort DR, Brown SJ.Sources of aminomethylphosphonic acid (AMPA) in urban and rural catchments in Ontario, Canada: Glyphosate or phosphonates in wastewater? Environ Pollut. 2015 Sep; 204:289-97.
Review	Coupe RH, Capel PD	2015	Coupe RH, Capel PD.Trends in pesticide use on soybean, corn andÂ cotton since the introduction of major genetically modified crops in the United States. Pest Manag Sci. 2015 Jul 20;
Not within scope/ exposure and monitoring	Hansen CT, Ritz C, Gerhard D, Jensen JE, Streibig JC	2015	Hansen CT, Ritz C, Gerhard D, Jensen JE, Streibig JC.Re-evaluation of groundwater monitoring data for glyphosate and bentazone by taking detection limits into account. Sci Total Environ. 2015 Dec 1; 536:68-71.
Not within scope/ecological and fate	Zhang L, Chen L, Liu F	2015	Zhang L, Chen L, Liu F.[Mutual Effect on Determination of Gibberellins and Glyphosate in Groundwater by Spectrophotometry]. Guang Pu Xue Yu Guang Pu Fen Xi. 2015 Apr; 35(4):966-70.
Not within scope/ecological and fate	Nguyen TH, Malone JM, Boutsalis P, Shirley N, Preston C	2015	Nguyen TH, Malone JM, Boutsalis P, Shirley N, Preston C.Temperature influences the level of glyphosate resistance in barnyardgrass (Echinochloa colona). Pest Manag Sci. 2015 Jul 23;
Not relevant	Chen JL, Tang W, Che JY, Chen K, Yan G, Gu YC, Shi DQ	2015	Chen JL, Tang W, Che JY, Chen K, Yan G, Gu YC, Shi DQ.Synthesis and Biological Activity Evaluation of Novel Î±-Amino Phosphonate Derivatives Containing a Pyrimidinyl Moiety as Potential Herbicidal Agents. J Agric Food Chem. 2015 Aug 19; 63(32):7219-29.
Not within scope/ecological and fate	Gaupp-Berghausen M, Hofer M, Rewald B, Zaller JG	2015	Gaupp-Berghausen M, Hofer M, Rewald B, Zaller JG.Glyphosate-based herbicides reduce the activity and reproduction of earthworms and lead to increased soil nutrient concentrations. Sci Rep. 2015 Aug 5; 5:12886.

Comments	Authors	PubDate (Year)	Full Citation
Not within scope/ecological and fate	Vannini A, Guarnieri M, BaÄ kor M, BilovÃ¡ I, Loppi S	2015	Vannini A, Guarnieri M, BaÄ kor M, BilovÃ¡ I, Loppi S.Uptake and toxicity of glyphosate in the lichen Xanthoria parietina (L.) Th. Fr. Ecotoxicol Environ Saf. 2015 Aug 3; 122:193-197.
Not within scope/ exposure and monitoring	Lupi L, Miglioranza KS, Aparicio VC, Marino D, Bedmar F, Wunderlin DA	2015	Lupi L, Miglioranza KS, Aparicio VC, Marino D, Bedmar F, Wunderlin DA.Occurrence of glyphosate and AMPA in an agricultural watershed from the southeastern region of Argentina. Sci Total Environ. 2015 Dec 1; 536:687-94.
Not within scope/ecological and fate	Zhao Y, Wendling LA, Wang C, Pei Y	2015	Zhao Y, Wendling LA, Wang C, Pei Y.Use of Fe/Al drinking water treatment residuals as amendments for enhancing the retention capacity of glyphosate in agricultural soils. J Environ Sci (China). 2015 Aug 1; 34:133-42.
Treatment for cattle and dogs	Qian W, Wang H, Shan D, Li B, Liu J, Liu Q	2015	Qian W, Wang H, Shan D, Li B, Liu J, Liu Q.Activity of several kinds of drugs against Neospora caninum. Parasitol Int. 2015 Dec; 64(6):597-602.
Not within scope/ecological and fate	Bakry FA, Ismail SM, Abd El-Atti MS	2015	Bakry FA, Ismail SM, Abd El-Atti MS.Glyphosate herbicide induces genotoxic effect and physiological disturbances in Bulinus truncatus snails. Pestic Biochem Physiol. 2015 Sep; 123:24-30.
Poisoning or incident	De Raadt WM, Wijnen PA, Bast A, Bekers O, Drent M	2015	De Raadt WM, Wijnen PA, Bast A, Bekers O, Drent M.Acute eosinophilic pneumonia associated with glyphosate-surfactant exposure. Sarcoidosis Vasc Diffuse Lung Dis. 2015 Jul 22; 32(2):172-5.
Review	Mesnage R, Defarge N, Spiroux de VendÃ´mois J, SÃ©ralini GE	2015	Mesnage R, Defarge N, Spiroux de VendÃ´mois J, SÃ©ralini GE.Potential toxic effects of glyphosate and its commercial formulations below regulatory limits. Food Chem Toxicol. 2015 Oct; 84:133-53.
Article retracted	Fluegge KR, Fluegge KR	2015	Fluegge KR, Fluegge KR.Glyphosate Use Predicts ADHD Hospital Discharges in the Healthcare Cost and Utilization Project Net (HCUPnet): A Two-Way Fixed-Effects Analysis. PLoS One. 2015; 10(8):e0133525.
Article retraction		2015	Retraction: Glyphosate Use Predicts ADHD Hospital Discharges in the Healthcare Cost and Utilization Project Net (HCUPnet): A Two-Way Fixed-Effects Analysis. PLoS One. 2015; 10(8):e0137489.
Review	Bonny S	2015	Bonny S.Genetically Modified Herbicide-Tolerant Crops, Weeds, and Herbicides: Overview and Impact. Environ Manage. 2015 Aug 22;

Comments	Authors	PubDate (Year)	Full Citation
Formulation/no glyphosate measurements	Mesnage R, Arno M, Costanzo M, Malatesta M, SÃ©ralini GE, Antoniou MN	2015	Mesnage R, Arno M, Costanzo M, Malatesta M, SÃ©ralini GE, Antoniou MN.Transcriptome profile analysis reflects rat liver and kidney damage following chronic ultra-low dose Roundup exposure. Environ Health. 2015 Aug 25; 14(1):70.
Not within scope/ecological and fate	Reed SE, Juzwik J, English JT, Ginzel MD	2015	Reed SE, Juzwik J, English JT, Ginzel MD.Colonization of Artificially Stressed Black Walnut Trees by Ambrosia Beetle, Bark Beetle, and Other Weevil Species (Coleoptera: Curculionidae) in Indiana and Missouri. Environ Entomol. 2015 Aug 4;
Not within scope/ecological and fate	Awuni GA, Gore J, Cook D, Musser F, Bond J	2015	Awuni GA, Gore J, Cook D, Musser F, Bond J.Seasonal Abundance and Phenology of Oebalus pugnax (Hemiptera: Pentatomidae) on Graminaceous Hosts in the Delta Region of Mississippi. Environ Entomol. 2015 Aug; 44(4):931-8.
Review/commentary	Faria MA	2015	Faria MA.Glyphosate, neurological diseases - and the scientific method. Surg Neurol Int. 2015; 6:132.
Not within scope/pest management and weed resistance	Maroli AS, Nandula V, Dayan FE, Duke S, Gerard P, Tharayil N	2015	Maroli AS, Nandula V, Dayan FE, Duke S, Gerard P, Tharayil N.Metabolic profiling and enzyme analyses indicate a potential role of antioxidant systems in complementing glyphosate resistance in an Amaranthus palmeri biotype. J Agric Food Chem. 2015 Sep 2;
Not within scope/ecological and fate	Balbuena MS, Tison L, Hahn ML, Greggers U, Menzel R, Farina WM	2015	Balbuena MS, Tison L, Hahn ML, Greggers U, Menzel R, Farina WM.Effects of sublethal doses of glyphosate on honeybee navigation. J Exp Biol. 2015 Sep; 218(Pt 17):2799-805.
Not within scope/ecological and fate	Mayakaduwa SS, Kumarathilaka P, Herath I, Ahmad M, Al-Wabel M, Ok YS, Usman A, Abduljabbar A, Vithanage M	2015	Mayakaduwa SS, Kumarathilaka P, Herath I, Ahmad M, Al-Wabel M, Ok YS, Usman A, Abduljabbar A, Vithanage M.Equilibrium and kinetic mechanisms of woody biochar on aqueous glyphosate removal. Chemosphere. 2015 Sep 1;
Not within scope/ecological and fate	Hagner M, Hallman S, Jauhiainen L, Kemppainen R, RÃ¤mÃ¶ S, Tiilikkala K, SetÃ¤lÃ¤ H	2015	Hagner M, Hallman S, Jauhiainen L, Kemppainen R, RÃ¤mÃ¶ S, Tiilikkala K, SetÃ¤lÃ¤ H.Birch (Betula spp.) wood biochar is a potential soil amendment to reduce glyphosate leaching in agricultural soils. J Environ Manage. 2015 Sep 3; 164:46-52.
Not within scope/ exposure and monitoring	Skeff W, Neumann C, Schulz-Bull DE	2015	Skeff W, Neumann C, Schulz-Bull DE.Glyphosate and AMPA in the estuaries of the Baltic Sea method optimization and field study. Mar Pollut Bull. 2015 Sep 2;

Comments	Authors	PubDate (Year)	Full Citation
Not within scope/ methods generating	Ross AR, Liao X	2015	Ross AR, Liao X.A novel method for the rapid determination of polyethoxylated tallow amine surfactants in water and sediment using large volume injection with high performance liquid chromatography and tandem mass spectrometry. Anal Chim Acta. 2015 Aug 19; 889:147-55.
Not within scope/ecological and fate	Fai PB, Mbida M, Demefack JM, Yamssi C	2015	Fai PB, Mbida M, Demefack JM, Yamssi C.Potential of the microbial assay for risk assessment (MARA) for assessing ecotoxicological effects of herbicides to non-target organisms. Ecotoxicology. 2015 Sep 11;
Poisoning or incident	Caloni F, Cortinovis C, Rivolta M, Davanzo F	2016	Caloni F, Cortinovis C, Rivolta M, Davanzo F.Suspected poisoning of domestic animals by pesticides. Sci Total Environ. 2016 Jan 1; 539:331-6.
Not within scope/ecological and fate	Song QY, Nan ZB, Gao K, Song H, Tian P, Zhang XX, Li CJ, Xu WB, Li XZ	2015	Song QY, Nan ZB, Gao K, Song H, Tian P, Zhang XX, Li CJ, Xu WB, Li XZ.Antifungal, Phytotoxic, and Cytotoxic Activities of Metabolites from EpichloÃ« bromicola, a Fungus Obtained from Elymus tangutorum Grass. J Agric Food Chem. 2015 Oct 1;
Not within scope/ methods generating	Gao X, Gu Y, Huang S, Zhen G, Deng G, Xie T, Zhao Y	2015	Gao X, Gu Y, Huang S, Zhen G, Deng G, Xie T, Zhao Y.Comparison of alternative remediation technologies for recycled gravel contaminated with heavy metals. Waste Manag Res. 2015 Sep 28;
Poisoning or incident	Lee JW, Hwang IW, Kim JW, Moon HJ, Kim KH, Park S, Gil HW, Hong SY	2015	Lee JW, Hwang IW, Kim JW, Moon HJ, Kim KH, Park S, Gil HW, Hong SY.Common Pesticides Used in Suicide Attempts Following the 2012 Paraquat Ban in Korea. J Korean Med Sci. 2015 Oct; 30(10):1517-21.
Not within scope/ecological and fate	Ahmad A, Negri I, Oliveira W, Brown C, Asiimwe P, Sammons B, Horak M, Jiang C, Carson D	2015	Ahmad A, Negri I, Oliveira W, Brown C, Asiimwe P, Sammons B, Horak M, Jiang C, Carson D.Transportable data from non-target arthropod field studies for the environmental risk assessment of genetically modified maize expressing an insecticidal double-stranded RNA. Transgenic Res. 2015 Oct 3;
Not within scope/ecological and fate	Napoli M, Cecchi S, Zanchi CA, Orlandini S	2015	Napoli M, Cecchi S, Zanchi CA, Orlandini S.Leaching of Glyphosate and Aminomethylphosphonic Acid through Silty Clay Soil Columns under Outdoor Conditions. J Environ Qual. 2015 Sep; 44(5):1667-1673.
Not within scope/ exposure and monitoring	Aris A, Leblanc S.	2011	Aris A, Leblanc S. 2011. Maternal and fetal exposure to pesticides associated to genetically modified foods in Eastern Townships in Quebec, Canada. Reproductive Toxicology. 31: 528-533.

Comments	Authors	PubDate (Year)	Full Citation
Not within scope/crop composition	Bohn T, Cuhra M, Traavik T, Sanden M, Fagan J, Primicerio R.	2014	Bohn T, Cuhra M, Traavik T, Sanden M, Fagan J, Primicerio R. 2014. Compositional differences in soybeans on the market: glyphosate accumulates in Roundup Ready GM soybeans. Food Chemistry. 153: 207-215.
Not within scope/ecological and fate	Cuhra M, Traavik T, Bohn T.	2014	Cuhra M, Traavik T, Bohn T. 2014. Life cycle fitness differencs in *Daphnia magna* fed Roundup-Ready soybean or conventional soybean or organic soybean. Aquaculture Nutrition. doi: 10.1111/anu.12199.
Review	Antoniou M, Habib MEM, Howard CV, Jennings RC, Leifert C, Nodari RO, Robinson CJ, Fagan J.	2012	Antoniou M, Habib MEM, Howard CV, Jennings RC, Leifert C, Nodari RO, Robinson CJ, Fagan J. 2012. Teratogenic effects of glyphosate-based herbicides: divergence of regulatory decisions from scientific evidence. Journal of Environmental and Analytical Toxicology. S4:006. doi: 10.4172/2161-0525.S4-006.
Microbiota/formulation/ no glyphosate measurements	Samsel A, Seneff S.	2013	Samsel A, Seneff S. 2013. Glyphosate's suppression of Cytochrome P450 enzymes and amino acid biosynthesis by the gut microbiome: pathways to modern diseases. Entropy. 15: 1416-1463.
Embryo exposures/formulation/no glyphosate measurements reported	Paganelli A, Gnazzo V, Acosta H, Lopez SL, Carrasco AE.	2010	Paganelli A, Gnazzo V, Acosta H, Lopez SL, Carrasco AE. 2010. Glyphosate-based herbicides produce teratogenic effects on vertebrates by impairing retinoic acid signaling. Chemical Research in Toxicology. 23: 1586-1595.
Formulation/small sample size	Seralini, GE, Clair E, Mesnage R, Gress S, Defarge N, Malatesta M, Hennequin D, Spiroux de Vendomois J.	2014	Seralini, GE, Clair E, Mesnage R, Gress S, Defarge N, Malatesta M, Hennequin D, Spiroux de Vendomois J. 2014. Republished study: long-term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize. Environmental Sciences Europe. 26: 14.