National Institute of Environmental Health Sciences
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Glyphosate and Glyphosate-Based Herbicides Cancer Hazard Assessment Protocol

Animal Cancer Studies

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Integrative Health Assessments Branch, NIEHS, NIH, HHS

About this report

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Note

This protocol was originally intended for internal cancer hazard assessments to reach level-of-evidence conclusions of carcinogenicity from animal and human cancer studies. Currently, we are refocusing the assessment for journal publications that assess and integrate the evidence, as well as discussing strengths, limitations, and research gaps. Thus, the steps related to reaching level-of-evidence conclusions will not be used in the manuscripts

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Introduction

Glyphosate is a synthetic compound and active ingredient in various products (e.g., glyphosate-based herbicides, [GBHs]) used as a nonselective systemic herbicide, particularly effective against perennial weeds, for commercial, residential, and agricultural purposes (ATSDR 2020). GBHs are the most widely used herbicide in the United States with substantial increases in total volume applied since the 1990s, in large part because of its use on genetically engineered herbicide-tolerant crops (Benbrook 2016). Since it was first registered as a pesticide (1974), over ~1.6 billion kilograms of glyphosate active ingredient have been applied in the U.S., representing ~19% of the estimated global use of glyphosate. As such, exposure to glyphosate is widespread, with >70% of U.S. National Health and Nutrition Examination Survey (NHANES) participants containing detectable concentrations of glyphosate from urinary biomonitoring sampling (Ospina et al. 2024).

The International Agency for Research on Cancer (IARC) classifies glyphosate as probably carcinogenic to humans (Group 2A) based on (1) sufficient evidence of carcinogenicity from studies in experimental animals and (2) limited evidence of carcinogenicity from studies in humans based on an association between non-Hodgkin lymphoma and glyphosate exposure. Mechanistic and other relevant data support the IARC cancer hazard classification. In contrast, other organizations have reached different conclusions. The U.S. Environmental Protection Agency (US EPA) has concluded that glyphosate is "not likely carcinogenic to humans (EPA 2020)," and the European Chemicals Agency (ECHA) concluded that the available evidence did not meet EU criteria to classify glyphosate as carcinogenic (ECHA 2023) based on their assessments of cancer studies in humans and experimental animals. Notably, there are differences in the agencies' missions, and what evidence was considered by each authoritative body and how the available data were evaluated (Portier et al. 2016). These assessments also did not include a recent large-scale cancer bioassay examining human-relevant doses of glyphosate and two formulations (Panzacchi et al. 2025).

To address the apparent discrepancy in hazard conclusions among authoritative bodies, and to incorporate the findings of the Panzacchi et al. study and other new studies, the Division of Translational Toxicology (DTT) is conducting a systematic review of cancer studies, including (1) animal cancer studies on glyphosate and GBHs and (2) human epidemiological studies of lymphohematopoietic cancers and GBH exposure. DTT will use methods outlined in the Report on Carcinogens (RoC) Handbook (2025) and apply the RoC listing criteria to the cancer hazard assessment to reach a draft hazard conclusion for each of the evidence streams separately.

This protocol draws on the general methods outlined in the RoC Handbook and provides specific methods used to conduct the cancer hazard assessment of the glyphosate and GBH animal cancer database.

Background Information

Glyphosate is produced in the United States as a technical-grade substance, with purity usually above 90%, although products with purity above 80% are also available. GBHs or other product on the market contain glyphosate concentrations ranging from 0.96% to 94%, and concentrations can vary substantially even within the same commercial product or trade name (ATSDR 2020).

The general public may be exposed to glyphosate through dermal contact with consumer products, crops, and contaminated soil; ingestion of plants, crops, and drinking water; and inhalation of mists during product use. The U.S. Food and Drug Administration's crop residue testing has shown high detection rates of glyphosate in crop products like corn and soybeans (up to ~59% detection); however, the concentrations of glyphosate are generally below EPA pesticide tolerance limits (US FDA 2025). Children may also be exposed through hand-to-mouth activities and by playing in contaminated soil. The highest potential for exposure to glyphosate is among agricultural workers, which occurs primarily through dermal contact; however, workers may also be exposed via inhalation, oral ingestion, and ocular contact. Glyphosate is not expected to bioaccumulate in the environment (ATSDR 2020).

Glyphosate is rapidly absorbed from the gastrointestinal tract and likely from the respiratory tract, but relatively little is absorbed through the skin. However, based on in vitro studies, surfactants present in certain GBHs can potentially increase dermal absorption and facilitate absorption through the skin and across the cell membrane. Once absorbed, glyphosate is distributed to tissues via the bloodstream, with no substantial accumulation in any specific tissue. Glyphosate is excreted primarily in the urine and feces, mainly as the parent compound, with only a small percentage metabolized (ATSDR 2020). In humans, the half-life of glyphosate in urine is less than 10 hours; thus urinary measures represent levels concurrent with the timing of sample collection (Connolly et al. 2019). Urinary glyphosate levels are higher among workers applying GBHs than non-working populations and are higher among workers not using gloves (ATSDR 2020; Chang et al. 2024).

1. Proposed Report Contents

The report will present the cancer hazard assessment for two evidence streams: human cancer studies and experimental animal studies. Evidence stream-specific conclusions will be reached, but not an overall conclusion. The evaluation does not include an assessment of cancer mechanistic studies.

- Overview (Chemical and exposure)
- Overview of Lymphohematopoietic Cancers (LHCs)
- Human Cancer Studies [LHCs]
- Animal Cancer Studies [all cancers]
- Discussion

2. Animal Cancer Assessment of Exposure to Glyphosate and Glyphosate-Based Herbicides

2.1. Background

2.1.1. Overall Objective

To reach conclusions about the level of evidence of the carcinogenicity of glyphosate and glyphosate-based herbicides (GBHs) provided by animal cancer studies based on the RoC listing criteria.

The assessment will be captured in a report, which will be peer-reviewed by several reviewers with toxicology, pathology, and statistical expertise and experience.

2.1.2. Protocol Contents and Evaluation Process

This document describes (1) the completed scoping and problem formulation steps used to identify key issues and develop the evaluation framework (Section 2.2), and (2) the proposed methods for conducting the evaluation, including approaches for assessing reporting quality (Section 2.3), study informativeness (Section 2.4), and evaluating and integrating the evidence (Section 2.5). The methods are based on applying the procedures outlined in the RoC handbook to the specific issues relevant to glyphosate and GBHs. The roles of the researchers (Appendix A) and the literature search terms (Appendix B) are described in appendices. The supplementary Excel file provides a list of the proposed included experimental animal studies.

Figure 2-1 provides a schematic of how the protocol (Step 2) fits into the cancer hazard evaluation process. The protocol is informed by scoping and problem formulation (i.e., developing the framework) done in Step 1, and the methods in the protocol are then used to conduct the cancer hazard evaluation and write the report (Step 3). Note that Steps 1 and 2 are iterative.

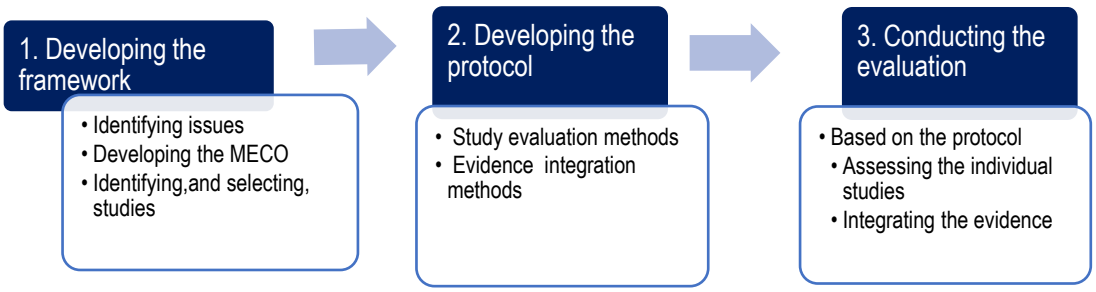

Figure 2-1. Cancer Hazard Evaluation Process

Figure 2-1 depicts the cancer hazard evaluation process. Scoping, problem formulation, and evidence mapping lead to the development of the framework (Step 1), which includes the overall objective and aims, MECO statements (i.e., body of evidence) to address the study objective(s), and identification of hazard-specific issues to be explored in the evaluation. This step has been completed, and the findings are reported in Section 2.2. This step also informed the methods, i.e., the protocol (Step 2) for conducting the

cancer hazard evaluation (Step 3), the results of which will be captured in the report. The methods focus on study evaluation (bias and study sensitivity, Section 2.4) and evidence integration (Section 2.5). **MECO** = **M**odel, **E**xposure, **C**omparison group, and **O**utcome.

2.2. Developing the Framework

Preliminary scoping and problem formulation activities were conducted to identify key issues for the assessment and identify and select the literature.

2.2.1. Glyphosate-Specific Issues

As mentioned in the introduction, several international and U.S. agencies have conducted cancer hazard evaluations or risk assessments on glyphosate, GBHs, or both. Our review of these reports, as part of our initial scoping activities, helped inform the identification of key scientific issues. An overview of these resources is provided in Table 2-1.

Table 2-1. Overview of Literature Resources for Glyphosate or Glyphosate-Based Herbicide Cancer Bioassays

Source	Year	Purpose	Comments
Agency for Toxic Substances and Disease Registry (ATSDR) Toxicological Profile	2020	Toxicological evaluation as required by law	Produces Minimal Risk Limits
European Chemicals Agency (ECHA)/European Union Food and Safety Authority (EFSA)	2021	Hazard and risk assessment used for re-registration of glyphosate as required by law, including pesticide tolerance levels	Access to animal cancer bioassay studies submitted under Confidential Business Information (CBI) by glyphosate registrants Original data tables for Panzacchi et al. (2025) study
U.S. Environmental Protection Agency (EPA)	2016	Hazard and risk assessment used for re-registration of glyphosate as required by law, including pesticide tolerance levels	Access to animal cancer bioassay studies submitted under Confidential Business Information (CBI) by glyphosate registrants
International Agency for Research on Cancer (IARC)	2017	Cancer hazard identification	Relied on publicly available information from secondary sources
Joint Meeting on Pesticide Residues (JMPR) Jointly sponsored by Food and Agriculture Organization of United Nations (FAO) and the World Health Organization (WHO)	2006; 2016	Toxicological evaluation	Review of experimental animal cancer studies submitted under CBI by glyphosate registrants
Greim et al.	2015	Journal review	Review of experimental animal cancer studies submitted under CBI by glyphosate registrants, with additional study information and original data tables

Source	Year	Purpose	Comments
Portier et al.	2020	Journal review	Statistical reanalysis of individual studies and across studies, with additional study information

Selection of Animal Cancer Studies in the Assessment

Many of the available cancer bioassays were conducted as submission requirements for the registration of glyphosate or its formulations. These studies are directly submitted to authoritative agencies (e.g., EPA, ECHA/EFSA) for evaluation. As such, the submitted cancer bioassays are subject to confidential business information, are not required to be made publicly available as a primary resource, and have generally not been published independently. However, these studies have been reviewed by regulatory bodies within the publicly available authoritative sources (see Table 2-1), which describe the methods and results with varying amounts of detail. Though this is considered a secondary resource, the level of description of these studies still provides an opportunity to independently assess the evidence, and many of these studies follow guideline requirements needed for submission. Moreover, our process includes a study informativeness assessment and thus can serve as an independent peer review of the studies. There is no consensus among the reviews on which studies to include and both reporting quality and biases or sensitivity concerns have been used to exclude studies. A key question is whether the reviews contain adequate detail to conduct a study informativeness evaluation of each study (see Section 2.2.3 for the methods to identify and select studies for inclusion).

Animal Models and Study Designs

All the glyphosate or GBH cancer bioassay studies were conducted in mice or rats and administered glyphosate in the diet (most) or drinking water, although glyphosate or GBHs were administered dermally in initiation/promotion or co-carcinogenicity studies. Animals could also be exposed dermally in the oral studies by self-grooming. Thus, the available database may be less informative for farm workers, who have the highest exposure levels in humans, primarily by dermal contact.

Two observational veterinary epidemiology studies were identified. Given the study designs and methods parallel human epidemiology studies, the study informativeness assessments for these studies will need to consider many issues similar to those in human epidemiology studies.

Lymphohematopoietic Cancers (LHCs)

LHCs are a heterogeneous grouping of blood- or lymph-based tumors, which include leukemias, lymphomas, and multiple myeloma. Several authoritative reviews have identified tumor sites of interest, and LHCs have been reported in some human cancer studies of glyphosate/GBHs. Many strains of mice develop lymphomas spontaneously in untreated controls and are sensitive to developing additional lymphomas in chemical carcinogenesis studies. Leukemias and lymphomas are rarely found in controls in most rat strains, with the exception of large granulocytic leukemia (e.g., mononuclear leukemia) in the Fischer rat, although both lymphomas and leukemias can develop in response to chemical treatments. There are sensitivity and other issues related to LHCs that may affect the study informativeness and evidence integration steps. We plan to include veterinary pathology expertise for LHCs to inform our assessment. As

mentioned in Section 1, the complete report will have a brief overview of LHCs in humans and animals.

The classification of LHCs in experimental animals has evolved over time in parallel with changes in human classifications (Ward 2006). For example, lymphomas used to be categorized as reticulum cell sarcomas. Limited reporting in older studies may miss lymphomas using different terminology.

Background incidences of lymphoma vary in strains/stocks such as B6C3F1 or C57BL/6 mice and vary by sex (higher in females). Background rates in CD-1 mice are lower than other strains or stocks. In CD-1 mice, rates are ~15% in females and ~5% in males. Two patterns of lymphoma induction in mice have been noted in NTP's database: (1) early induction leading to decreased survival, usually involving T-cell lymphocytes, and (2) increased incidence after 18 months to 2 years, which are usually B-cell lymphomas (Ward 2006). Retrovirus (i.e., mouse leukemia virus) can cause lymphomas and may interact with chemical carcinogens to cause thymic lymphomas in CD-1 mice. Ideally, studies should provide information on the cell type of lymphoma.

Lymphomas are one of the most prevalent tumors in dogs, and multicentric lymphomas (MCL) are the most common lymphomas, comprising 84% of all canine lymphomas (Rocha et al. 2025). Similar to humans, they mainly consist of B-cell, T-cell or mixed T and B-cell lymphomas. MCL involve many lymph nodes and may also affect the spleen, liver, or bone marrow. MCL is more common in certain breeds of dogs such as Dobermans, Boxers, and Golden Retrievers; incidence also varies by geographical location and age (Dobson 2013; Comazzi et al. 2018).

Statistical Analyses

Several assessments or reviews have reanalyzed or conducted analyses of the tumor data when not provided by the study authors, including pairwise tests, trend tests, and tests of significance vs historical controls. Portier et al. (2020) also conducted pooled analyses across studies for certain types of cancers in animals. Some of these approaches used less traditional statistical methods. To inform the assessment, we have engaged experts in animal cancer study statistical methods to independently evaluate the statistical approaches used for both individual studies and across studies.

2.2.2. Overall Assessment Approach

Our goal is to include all the relevant studies in the assessment, including studies submitted for registration and those in the peer reviewed literature. As mentioned previously, limited access to details in the regulatory studies may complicate the study informativeness evaluation. Rather than excluding studies or rating them as potentially biased based on lack of reporting, we aim to stratify studies by reporting quality as well as by study informativeness. Figure 2-2 provides an overview of our strategy, and the individual steps are discussed in Sections 2.2.3, 2.3, 2.4, and 2.5.

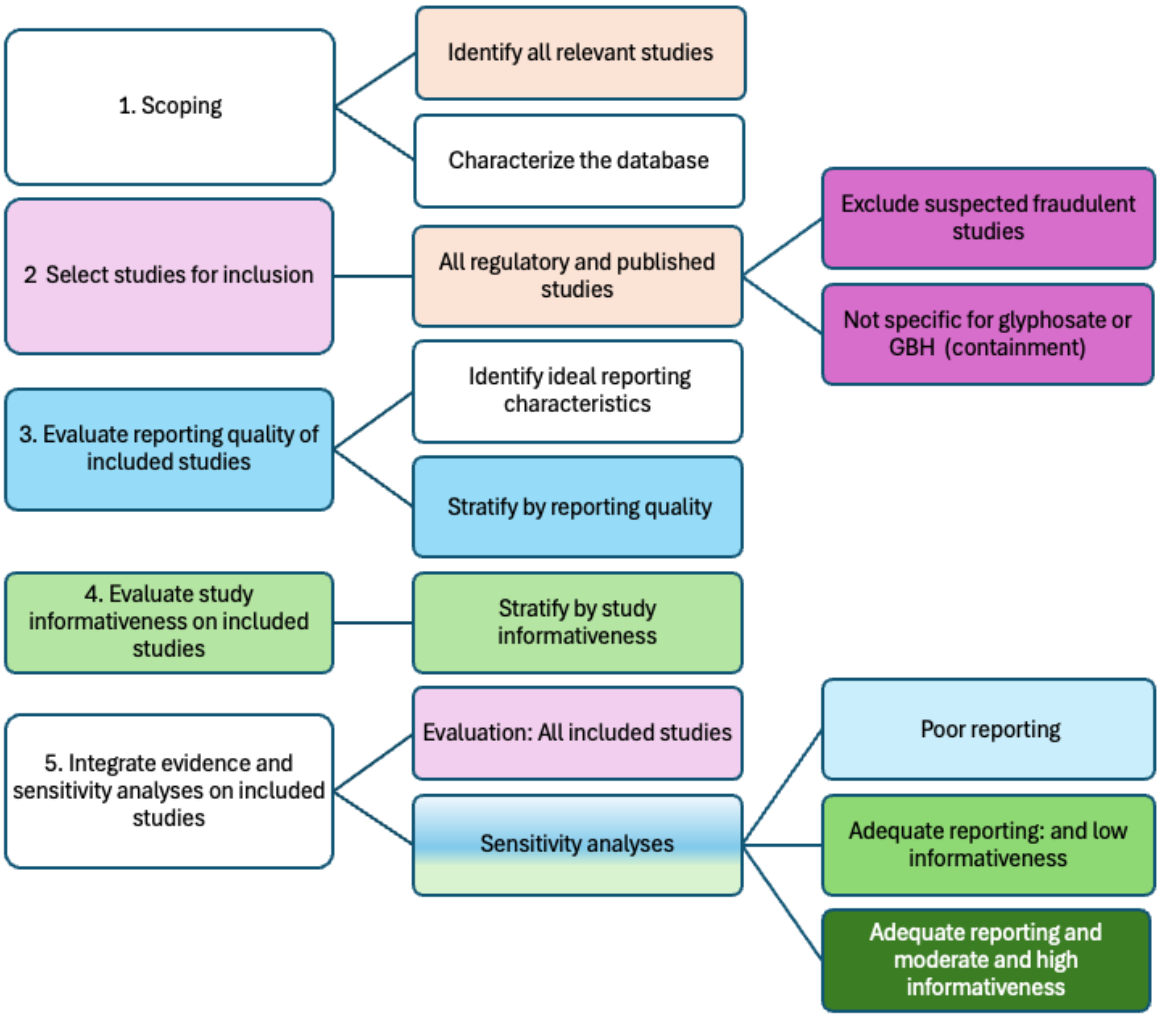

Figure 2-2. Strategy for Conducting the Evaluation of Glyphosate Studies in Experiment Animals.

Figure 2-2 presents the evaluation strategy. Studies are identified from biomedical citation searches and authoritative reviews (step 1). All relevant studies identified in step 1 were included except some studies with critical concerns of fraudulence (step 2). Next, studies are assessed for their reporting quality (step 3) and informativeness (potential for bias and study sensitivity). Then, the findings are evaluated considering any bias, and the evidence is integrated across studies. Lastly, a sensitivity analysis (step 4) is performed by stratifying evidence by reporting quality (if possible) and study informativeness.

2.2.3. Study Identification and Selection

The body of evidence is defined by the MECO or PECO (**M**odel or **P**opulation, **E**xposure, **C**omparison Group, **O**utcome) Statements (Table 2-2). Studies were identified from the peer-reviewed literature and authoritative organizations (registry studies).

Table 2-2. Initial MECO/PECO

Element	Criteria
Model/Population	Experimental animal cancer studies: Any experimental animal cancer models and species exposed longer than half their lifetime (e.g., ~1 year for rodents), unless tumors are observed in 1-year studies, the studies use sensitive models, or they are co-carcinogen or initiation/promotion studies
	Observational veterinary cancer studies: Any species/population
Exposure	Glyphosate alone (active ingredient), glyphosate-based herbicides (e.g., glyphosate formulations), glyphosate metabolites (e.g., AMPA, MPA)
Comparison group	No or low exposure to glyphosate, but comparable study design with exposure to vehicle only or in combination with a carcinogen (e.g., initiation/promotion, or co-carcinogen studies)[a]
Outcome	Tumors at any organ site

[a]Co-carcinogen or initiation/promotion studies will be considered as supporting, but not direct evidence.

Abbreviations: AMPA = Aminomethylphosphonic acid, MPA = Methylphosphonic acid

Registration Studies

As noted above, most glyphosate animal cancer studies were conducted for pesticide registration purposes and have been reviewed under confidentiality agreements by several authoritative organizations, but most have not been published independently in the peer-reviewed literature. However, these authoritative sources have described, in varying levels of detail, the methods and results of each study.

Given the nature of this unique database of studies, we are amending our typical approach (RoC Handbook) for study identification and inclusion. Our amended process for selecting studies for inclusion in this assessment involved two steps: (1) identifying studies reviewed by authoritative organizations and selected reviews and (2) characterizing the overall study database using the cumulative data for each study from all sources. Therefore, we will be relying on the totality of reported data on a given study from multiple sources, rather than rely on one source. As mentioned above, our goal is to consider all potential studies and do separate evaluations of reporting and study informativeness, which can be used in sensitivity analyses. To assess reporting quality, we present a list of elements representing an ideal level of reporting below (section 2.3).

Identifying the Registration Studies

As mentioned above, most glyphosate animal cancer studies were conducted for pesticide registration purposes and have been separately reviewed by several authoritative organizations but were not independently published in peer-reviewed journals. We searched the sources listed on Table 2-1 – including five assessments by authoritative organizations and two comprehensive journal reviews – to identify glyphosate and glyphosate-based herbicide (GBH) animal cancer bioassays. We included these journal reviews because they provide additional primary information on the individual studies. Authoritative organizations were identified from the general sources listed in the RoC Handbook.

Characterization of the Database

Several issues arose in our scoping of each source described above. First, we noted a lack of consistency in the number of studies evaluated across authoritative sources. Some registration studies were only described by one authoritative source, whereas other registration studies were described by some or all. Second, there was a lack of consistency in the level of detail for study descriptions across the available sources. Third, given that the registration studies are subject to confidentiality by some authoritative bodies (e.g., EPA, EFSA), each study was labeled differently across each source. Nomenclature included the primary author, the primary lab or company, an assigned code given by an authoritative body, or the date the study was conducted. Lastly, we note that an authoritative source may have described a specific study but deemed a study unacceptable according to their own criteria.

To ensure completeness of this complex database, we did the following:

- Identified every available study across all authoritative sources, using the primary author as the study identifier (this was the most common nomenclature across the sources). Other study labels were also captured.
- For each study, we noted which authoritative sources described the study, if a study was included in their evaluation, and the page numbers within each authoritative source.
- Extracted key study methods from each description, including study duration, rodent strain, dose concentrations, number of animals per group, dose substance, dose purity, and if a laboratory or company was given. Relevant study information was key to matching up the studies across sources.
- For each study, we collated all available study information into a single study document to evaluate the totality of available information of a given study.

This activity allowed us to thoroughly capture all available information prior to the study evaluation phase of our assessment.

Biomedical Database Searches

Biomedical citation databases, namely PubMed, Scopus, and Web of Science, were searched for animal cancer studies and exposure to glyphosate and glyphosate based herbicides (GBHs) by combining search terms for glyphosate (see Appendix A), cancer (see RoC Search String Document), and animal cancer studies (see RoC Search String Document) using the procedures outlined in the RoC Handbook.

Search results were processed in Endnote and imported into a content management system [e.g., Health Assessment Workplace Collaborative (HAWC)] software to select relevant literature (Shapiro et al. 2018). Studies are included in the review if they meet the requirements of the initial MECO. Searches will be done periodically for any retracted studies.

Study Selection and Overview of Included Studies

Three registration studies identified in our broad search were excluded based on critical concerns independent of reporting and study informativeness (see Table 2-3). An early version of the study reported by Séralini (2012) was retracted (Editor-in-Chief retraction 2014) and later resubmitted to another journal (Seralini et al. 2014) as there was controversy over the rationale

for its retraction (Resnik 2015). We are including the 2014 publication as per our aim to be inclusive and our process to rigorously assess study informativeness.

Table 2-3. Excluded Studies and Rationale

Study	Exclusion rationale
Reyna and Gordon (1973)[a] Reyna and Gordon (1973a)[a]	EPA noted these two registration studies from the same lab were suspected of containing falsified or fabricated data
Burnett et al. (1979)[a]	EPA retracted this study as the test chemical was a contaminant of glyphosate (N-nitrosoglyphosate), and not glyphosate or a GBH

[a]As cited in EPA 2016

Table 2-4 to 2-6 present an overview of the animal cancer studies evaluating glyphosate and GBHs (see Supplementary data for the full list of studies). Based on a preliminary scoping review, we identified 20 rodent cancer bioassays of glyphosate exposure: 11 studies in rats (mostly outbred strains) and 9 in mice (mostly outbred strains). In all but two studies, glyphosate was administered in the feed. In the rat drinking water study, exposure was initiated in utero via maternal exposure. The mouse drinking water study used transgenic mice. In addition, one co-carcinogenicity study was identified in which mice were exposed to glyphosate in drinking water in combination with ultraviolet radiation (UVR) (Lerche et al. 2025).

For GBHs, seven animal cancer studies were identified. These included five oral exposure bioassays conducted in mice and rats, as well as two initiation–promotion (George et al. 2010) or co-carcinogenicity studies (Lerche et al. 2025) in mice involving dermal exposure. One bioassay also fed animals with GBH-treated crops in addition to testing the GBH directly (Seralini et al. 2014).

Table 2-4. Cancer Bioassays of Glyphosate and Glyphosate-Based Herbicide Exposure

Route	Species		Strain		Publication Type	
Glyphosate exposure						
Diet	Rat	10	Sprague Dawley	5	Registration	10
			Wistar	4		
			Fischer	1		
Drinking + in utero	Rat		Sprague Dawley	1	Peer Rev Lit	1
Diet	Mouse	8	CD-1	5	Registration	8
			Swiss Albino	1		
			Balb/c	1		
			CFLP	1		
Drinking water	Transgenic mouse	1		1	Peer Rev Lit	1

Route	Species		Strain		Publication Type	
Glyphosate based herbicides exposure						
Feed	Rat	2	Sprague Dawley	2	Registration	1
					Peer Rev Lit	1[a]
Drinking water + in utero	Rat	1	Sprague Dawley	1	Peer Rev Lit	1
Drinking water	Rat	1	Wistar	1	Peer Rev Lit	1
Feed	Mouse	1	CD-1	1	Registration	1

[a]One study also fed rats maize treated with GBHs

Table 2-5. Co-Carcinogenicity or Initiation Promotion Studies in Mice of Glyphosate or Glyphosate-Based Herbicide Exposure

Exposure	Route of exposure		Strain/ stock		Publication type
Glyphosate	Drinking water	1	Hairless	1	Peer Rev Lit
GBH	Dermal	2	Hairless	1	Peer Rev Lit
			Swiss albino	1	Registration

We also identified two veterinary epidemiology studies evaluating exposure to GBHs in dogs. These included a case–control study nested within the Golden Retriever Lifetime Cohort Study that used banked biological samples (Tindle et al. 2025), and a case–control study in boxers that incorporated a cross-sectional analysis (Braman et al. 2025). Both studies evaluated associations with multicentric lymphoma (MCL).

Table 2-6. Veterinary Epidemiological Studies of Dogs Exposed to Glyphosate-Based Herbicides

Breed	Study design	Exposure assessment
Boxers	Case-control: multi-centric lymphoma (MCL)	Urine sampling Environmental sampling: Drinking water
Golden Retrievers	Nested case control study: Multi-centric lymphoma (MCL) Golder Retriever Lifetime Cohort Study	Banked urine samples

2.3. Reporting Quality Assessment

Using the collective data for each study (e.g., across sources), each experimental animal study will be evaluated for reporting quality (Table 2-7). In general, studies with poor reporting quality, e.g., those for which we are unable to evaluate the potential for bias or sensitivity, will be considered separately in the sensitivity analyses (if relevant) and will not be grouped with

studies with potential biases based on adequate reporting (see Figure 2-2). Note that the reporting quality is based on the secondary source and does not necessarily reflect the quality in the report submitted to the regulatory agency.

Ideally, the authoritative source or a study should have adequate detail for the study informativeness domains (see Section 2.4). We note that many peer-reviewed studies (especially older studies) often have sparse details on methods and results.

Table 2-7. Ideal Reporting Information.

Domain	Ideal information
Model	Species, strain, sex, and number of treated animals and concurrent controls
	Historical control data
Exposures	Chemical purity
	Regimen: Dose (mg/kg/day, concentration in feed, preferably internal dose), route, duration,
Conduct	Randomization procedures, husbandry, pathological evaluation
	Study duration
Outcome methods	Pathology/necropsy methods, including tumor type, and tissue examined
Findings	Survival and body weight (ideally data over multiple time points)
	Non-neoplastic lesions
	Tumor incidence, multiplicity, and time to tumor
	Tumor type (malignant vs. benign reported separately), cell type
Statistics	Pairwise or trend, or information to calculate pair-wise p-values

2.4. Study Informativeness Assessment of Individual Studies

The methods are adapted from the RoC Handbook (NTP 2025). Each primary study is systematically evaluated for its ability to inform the cancer hazard evaluation using five domains related to bias – study design, exposure conditions, outcome assessment, potential confounding, and analysis – and one domain related to study sensitivity [or the ability of the study to detect a true effect (Cooper et al. 2016)] that includes elements related to study design and exposure conditions. These 'signaling questions' highlight concerns toxicologists usually consider when evaluating study informativeness and are used to increase transparency but are not meant to be a checklist. The potential for a given bias in a study does not necessarily or automatically mean that the findings of the study should be disregarded. When adequate information is available, the direction of the bias (away or towards the null) should be considered. In answering each question on whether there is a potential bias or limitation, reviewers provide their judgment by comparing the study elements with those of an *ideal* study for a specific endpoint. *Ideal* study elements have no to minimal concern for potential bias and are sensitive enough to detect an effect if present (See Table 2-8 and Table 2-9 for glyphosate or GBH specific guidance on assessing biases for each question). Differences in reviewer judgments are resolved by discussion between the reviewers. A small subset of studies may be used in a "pilot" phase to discuss and resolve any

ambiguity before proceeding with evaluation of the full set of studies. Study authors may be contacted by reviewers to obtain additional information needed for our evaluation. Reporting quality may also be noted (e.g., missing information).

Response to signaling questions

- No/Minimal concerns: Study design or methodologies are ideal or very close to the ideal study characteristics and potential for bias is unlikely or minor. These studies are generally considered informative for the cancer hazard evaluation.
- Some concerns: Study design or methodologies indicate a possible low to moderate risk of bias. These studies are generally considered informative for the cancer hazard evaluation.
- Major concerns: Study designs or methodologies suggest that the potential for a specific type of bias is high. However, depending on the direction and distortion of the potential bias, the study may still be informative for cancer hazard evaluation, but should be viewed with caution.
- Critical concerns: Study design or methodologies suggest that the bias would likely make study findings unreliable for hazard identification. This category is rare.
- No information in the study: The information in the study is inadequate to evaluate the level of concern.
- ***Direction of bias:*** ↑ Away from the null or overestimate of the effect (e.g., false positive); ↓ towards the null or underestimate of the effect (e.g., false negative); not known (↔ unable to determine).

2.4.1. Study informativeness Guidance

Study informativeness guidance for experimental animal studies and veterinary epidemiology studies are presented in Table 2-8 and Table 2-9.

The study informativeness for experimental studies encompasses five domains: study design, exposure bias, outcome bias, confounding, and statistics. Study sensitivity integrates study model, dose, statistical power, and study duration.

The study informativeness assessment for veterinary epidemiology studies encompasses five bias domains–selection bias, exposure misclassification or mismeasurement, outcome misclassification, confounding, and analyses–and a study sensitivity domain (Table 2-9).

Table 2-8. Study Informativeness Guidance: Experimental Animal studies

	Signaling question	Guidance	Response options
Study Design Bias	**Randomization** Is there concern that the methods of randomization of animals prior to treatment were inadequate?	No judgment will be made for the domain based on the absence of reporting. If performed after 1978, registration studies are likely GLP studies, and randomization would most likely be typical. Some studies may employ less comprehensive methods (e.g., ensuring equal body weight across groups), which are considered adequate but are less ideal than true or full randomization.	***Minor concerns*** Animals are randomized to control and experimental groups. ***Some/Major concerns*** Randomization was conducted but there are concerns about the adequacy of the methods or were not fully randomized. ***Critical concerns*** There is direct evidence that randomization of animals to dose groups did not occur.
	Controls Is there concern that the concurrent control group is not adequate for evaluating effects across treatment groups?	Concurrent controls are considered to be the most relevant comparison group for evaluating potential exposure-related tumor effects. Ideally, the concurrent control group included at least as many animals as did each treatment group.	***Minor concerns*** Controls are treated as similar as possible to the exposed animals but without the test substance, e.g., appropriate vehicle controls. ***Some concerns*** Concurrent controls but some concerns about similarity with (or have substantially fewer animals than) exposed groups. ***Major concerns*** No concurrent controls were used; historical controls are available. ***Critical concerns*** No concurrent or relevant historical controls (that could serve as surrogate controls) are available.

	Signaling question	Guidance	Response options
Exposure bias	**Dose selection** Is there concern that the dose level was too high (e.g., exceeds the maximum tolerated dose)?	The authors should state their rationale for dose selection, and it would ideally be based on a separate pre-chronic study. Exceedance of the maximum tolerated dose involves an evaluation of multiple indicators of overt toxicity. Examples of indicators include substantial treatment-related decreased body weight or survival (not related to tumors), especially early in the study, and clinical signs of overt toxicity.	***Minor concerns*** Minimal treatment-related effects on body weights or survival (unless related to tumors). ***Some concerns*** Reduced survival due to toxicity, palatability or other situations (e.g., due to fighting), but not substantially reduced. ***Major to critical concerns*** Severe toxicity in all treatment groups is seen. Survival due to toxicity is reduced sufficiently to affect statistical power. Reduced survival due to tumors is not a concern.

	Signaling question	Guidance	Response options
Sensitivity and design	Is there concern that the study design (i.e., animal model, number of animals/dose group and control group, study duration, dose(s) and exposure conditions) is sensitive enough to adequately detect a neoplastic effect if present? This question considers • Animal model • Statistical power (number of animals/group) • Study duration • Exposure regimen: duration and dose level	Ideally, adequate number of rodents should be exposed and observed for most of their lifespan (~2 years). Shorter duration studies may be adequate for sensitive model strains or for initiation/promotion studies but requires justification. As mentioned in the MECO statement, chronic or sub-chronic toxicity studies less than a year are generally excluded. No preliminary concerns were found for the animal models as most studies used outbred stocks or inbred strains with intermediate sensitivity to carcinogenicity. However, the assessment will consider tumor specificity, including tumor rarity or high or variable background rates, for different models. In general, 50 animals per dose group is considered sufficient for detection of most types of tumors given adequate doses. Higher numbers of animals may be required to detect rare tumors or when using low (sub MTD) doses. Ideally, studies should include multiple groups exposed to different dose levels, and time to first tumors. Controls are most likely exposed to low levels of glyphosate found in rodent feed. This may be more of a concern for studies of lower doses. In addition, the accuracy of nominal treatment concentrations could be altered by adsorption of glyphosate to test materials, especially glass, used for storage, preparation, or administration of glyphosate solutions.	***Minor concerns*** Glyphosate studies in strains/stocks of at least intermediate sensitivity using conditions close to the ideal conditions (defined in guidance). ***Some concerns*** Models designed to detect only adenomas or limited cancer sites) otherwise conducted close to the ideal study. Other studies may have less than ideal conditions for some but not all aspects of the study design or exposure conditions (e.g., duration, number of animals). ***Major concerns*** Insensitive model or inadequate conditions for the animal model (see guidance).

	Signaling question	Guidance	Response options
Outcome bias	Is there concern that the methods used to assess tumor outcome (necropsy, gross pathology, histology, or diagnosis) are not adequate to attribute the effects to the exposure?	Ideally, each study should include full gross necropsies of all tissues and histopathological examination of most of them, with similar methods used for control and treated animals. If details of the histopathological examination (e.g., cell type, preneoplastic lesions) are not reported, at a minimum tumor type (and whether benign or malignant) should be reported. Although blinding generally is considered important to reduce bias in the assessment of subjective outcomes (such as behavior), nonblinding may be preferred for cancer outcomes, to determine normal background histology. The NTP uses an informed approach to histopathological evaluation in its toxicity and carcinogenicity studies (Sills et al. 2019). This principle applies to NTP studies, provided that the necropsy and histology methods used were adequate and consistent. LHC: The classifications of leukemias and lymphomas in experimental animals have evolved over 50 years to parallel changes in human classification (Ward 2006). For example, some lymphomas used to be categorized as reticulum cell sarcomas. Ideally lymphomas should be categorized as T-cell or B-cell. Renal tumors: Ideally, additional sectioning of kidneys should be carried out if tumors are observed in the standard initial limited sections.	***Minor concerns*** Traditional models: Complete necropsies and gross pathology are reported for all tissues and histopathology examination done on most tissues for both controls and treated groups. Models for one tumor type: Histopathology conducted for tumor site of interest. ***Some concerns*** Benign and malignant tumors but insufficient identification of cell type or preneoplastic lesions reported**.** Complete histopathology only conducted on select dose groups or tissues. ***Major concerns*** Histopathology is not done to confirm or characterize suspected neoplasms. ***Critical concerns*** Controls and treatment groups analyzed differently to the extent it would compromise the study interpretation

	Signaling question	Guidance	Response options
Confounding	Is there concern for potential confounding? What is the relative impact of the confounding?	Sources of potential confounding in animal studies are the use of an impure chemical that contains other potential carcinogens, inadequate animal husbandry conditions, and lack of monitoring for pathogens that may be linked to cancer. Specific Pathogen Free (SPF) animals purchased from reputable sources, along with periodic colony monitoring at the study laboratory decreases concern for infections. Purity of the glyphosate in most registration studies is ~95% or higher. GBHs typically contain at least 40% or higher glyphosate, although GBHs with lower glyphosate levels are also marketed.	***Minor concerns*** The study uses relatively pure (~95%) glyphosate or well characterized GBH, adequate animal husbandry conditions, and pathogen testing, or purchased animals from reputable sources (e.g., disease free). ***Some, Moderate/Major concerns*** Potential for bias depends on documentation of lower glyphosate purity, poor husbandry and co-exposures. Most studies will not report on animal husbandry and will not be downgraded unless there are proxies to suggest poor husbandry. ***Critical concerns*** Strong evidence that poor animal husbandry conditions will substantially compromise interpretation of the findings and there are no data to evaluate the extent of the confounding. Strong evidence of carcinogenic impurities present in administered dose at significant concentrations.

	Signaling question	Guidance	Response options
Statistics and analyses	Is there concern that different types of tumors were inappropriately combined in the analysis?	Analyses of benign and malignant tumors from the same tissue type should be reported both separately and combined. All lymphomas diagnosed in different organs in each animal should be combined by the study authors and reported as a single neoplasm per individual animal.	***Minor concerns*** Tumor types of the same cellular origin are reported both separately as well as combined malignant and benign tumor types in the analysis. ***Some concerns*** Tumors of different cellular origin are reported individually but may also be inappropriately combined. However, it may be possible to conduct appropriate *ad hoc* analysis. ***Major concerns*** Tumor types of different cellular origins are combined, or tumors are only specified for a particular organ, as benign or malignant without reporting their cellular origin.

	Signaling question	Guidance	Response options
	Is there concern that statistical analyses are inadequate or were not conducted for evaluating the results? If statistical analyses are not conducted, are the results reported in sufficient detail for *ad hoc* analysis?	Both pairwise and trend tests should be reported. If not, the study should at a minimum present incidence data for specific tumors, so that statistical tests can be performed. Ideally, the studies should use survival-adjusted statistical methods, such as the poly-3 test (Bailer and Portier 1988), to accommodate differential survival across exposed groups. If survival is not a concern, the Cochran-Armitage test is appropriate. If there is a treatment related survival effect (e.g., higher mortality in treated animals), the Cochran-Armitage P value will be higher than the survival adjusted p value. Conversely, it would be lower than the survival adjusted p value if there is higher mortality in the controls. Multiple-comparison adjustment is not needed because we conduct a weight of evidence approach to evaluate biological significance and an analysis of NTP showed the false positive rate to be low. Statistical reanalysis using non-traditional methods will be reviewed by experts in bioassay statistics.	***Minor concerns*** The study reports appropriate methods of analysis using relevant data. Analyses are adjusted for survival when relevant. ***Some concerns*** Appropriate analyses are conducted but are not adjusted for survival and there are survival concerns. ***Major to critical concerns*** There is strong evidence that reporting of data and analytical methods are so limited that the findings are not interpretable.

LHC = Lymphohematopoietic cancers

Table 2-9. Study Informativeness Guidance: Veterinary Epidemiology Studies

	Signaling question	Guidance	Response options
Selection bias	Is there a concern that selection into (or out of) the study was related to both exposure (e.g., GBH) and cancer? Are there concerns that selection methods are not adequate? Do the eligibility criteria or recruitment strategies differ for study participants (pet owners and dogs) such as for cases and controls or exposed and unexposed? Is there concern about attrition bias or incomplete follow-up?	Ideally, the groups should be similar in all respects except for exposure status (cohort) or disease status (case-control), and from the same underlying population. Participation should not be related to both outcome and exposure status. Cases and controls should be selected from the same underlying population. For cohort (and nested case-control), the cohort should be clearly defined and no evidence that follow-up differs between exposed and unexposed.	***Minor concerns*** Cases and controls, or exposed and unexposed, were selected from the same population by similar methods and criteria. There is no evidence that selection of the participants was related to both GBH exposure and cancer. ***Some/Major concerns*** There is some evidence that study selection may be related to both exposure and outcome. ***Critical concerns*** There is substantial evidence that selection or attrition of participants was clearly related to GBH exposure and outcome.
Exposure Misclassification	Is there concern that the exposure assessment did not distinguish between exposed and non-exposed animals or among exposure categories at a relevant time window of exposure? • Is there concern that the **exposure proxy** did not adequately represent the exposure of interest at an appropriate time window of exposure for the outcome of interest? • Is there concern about error in measurement of the exposure of interest or exposure proxy? • Is any misclassification differential or nondifferential, and what is the predicted direction or distortion of the	Ideally, the biomarker should be a valid biomarker of cumulative exposure measured at a relevant exposure window (and prior to disease). Caution should be taken with using short-term or nonpersistent exposure biomarkers in the context of cancer, which has a long latency, unless there are multiple longitudinal measures or evidence suggesting chronic, consistent exposure. Biomarkers should be sampled in relevant tissue, at relevant times, and measured using assays with adequate limits of detection and quantification. Glyphosate has a short in vivo half-life (less than 10 hours) and its use varies by season and geographically (e.g., urbanicity, north vs. south). Within a season, its use could vary on a daily or weekly basis. Exposure levels in dogs could depend on timing when the dog was outside vs. human application of glyphosate or proximity of use in nearby agriculture farms.	***Minor concern*** The exposure proxy closely approximates the exposure of interest (glyphosate) and is assessed at multiple times for a relevant time window. Any measurement error is non-differential and small. Environment monitoring samples are taken at multiples times at relevant time windows. ***Some concern*** Study uses an appropriate proxy measure assayed at several times, but the number or timing of samples (biomarkers or environmental samples) are less than ideal, or the assay conditions are not ideal. Any measurement error is non-differential ***Moderate/Major concern*** Exposure proxy does not capture the exposure well or in the relevant time window, or

	Signaling question	Guidance	Response options
	effect estimate (if there is adequate information)?	Reverse causation (presence of a tumor causes changes in urinary glyphosate) may be less of a concern for glyphosate as the parent compound (not the metabolite) is measured. However, urinary bladder lymphomas (if any) could affect urinary excretion. Environmental monitoring (e.g., tap water) measures the exposure directly and should be measured in a relevant time window of exposure The median half-life of glyphosate in tap water is a few days up to 90 days depending on environmental conditions. Glyphosate in tap water usually comes from nearby agricultural use and is of more concern in areas using well water. Integrating different exposure metrics (e.g., environmental exposure monitoring and biomarkers) can be considered a good exposure proxy.	exposure categories overlap. Minimal additional exposure metrics are available. ***Critical concern*** Exposure assessment is not at the individual level, or the exposure proxy does not approximate glyphosate, the exposure of interest, and there is strong evidence that it is unable to differentiate between exposed and unexposed.
Outcome Misclassification	Is there a concern that the outcome measure does not reliably distinguish between the presence or absence of the cancer under study?	Ideally, studies will measure cancer incidence. Cases should be histologically or cytologically confirmed and ideally should be assayed for cell type (B vs. T). Multi-centric lymphoma is often fatal if untreated. If treated, remission is relatively high, but long-time survival can be low (<10%) and depends on many factors such as the type and grade of the tumor.	***Minor concerns*** Cancers are histologically/cytologically verified by a reliable source. ***Some concerns*** Cancer diagnoses are reported in record databases but not histologically/cytologically verified. ***Moderate/Major concerns*** Cancer diagnosis and type are self-reported by pet owners, and neither are verified by cancer registry or medical/veterinary hospital records. ***Critical concerns*** There is strong evidence that cancer diagnoses are likely related to exposure status.

	Signaling question	Guidance	Response options
Confounding	Is there concern for potential confounding?	Ideally, studies should consider confounders in the analysis. Confounders should be assessed by interview or questionnaire of the pet owner, or through environmental or biomonitoring (co-exposures). Potential confounders are risk factors that are thought to be associated with exposure. These include: • Pet: sex, age, and age at diagnosis • Pet owners: SES, other herbicides or pesticides Other risk factors for lymphoma in dog–exposure to volatile organic compounds found in household solvents, paint and other products are not thought to be highly correlated with glyphosate exposure. Environmental tobacco smoke is not considered to be a risk factor for lymphomas.	***Minor concerns*** The study measured all major potential confounders and used appropriate statistical analysis or designs. ***Some/Major concerns*** Potential for bias depends on the degree by which the study addressed the major concerns, including how thoroughly they were addressed (statistical analysis vs. external information), and the number and importance of the confounders. ***Critical concerns*** There is strong evidence that the effects of the exposure cannot be distinguished from the effects of the potential confounders.
Analysis	Is there concern about whether the data assumptions used in the statistical analysis were adequate? Is there concern that statistical analyses were inadequate or were not conducted for evaluating the results?	The study used relevant data and appropriate assumptions and analysis methods. The analysis used an appropriate method to correct for urinary dilution (e.g., creatinine correction or adjustment). The analysis uses appropriate statistical model such as logistic regression. If a matched case-control study, conditional logistic regression is used, and matching is adequately described. Control for variables not related to exposure or disease will likely reduce the precision of the risk estimate. There should be little or no concern that data are missing for reasons related to exposure or disease.	***Minimal or some concern*** The study used relevant data and appropriate assumptions and analysis methods. The study corrected for urinary dilution. Matching is well described, and appropriate statistical methods are used. ***Some or major concern*** The study used relevant and appropriate data and methods, but did not correct for urinary dilution, or the study did not use ideal methods, but the results can still be interpreted. Analytic methods for matching not well described. There is a concern for missing data. ***Critical concern*** There is strong evidence that the study's analytical methods were so limited that the

	Signaling question	Guidance	Response options
			findings were uninterpretable or were distorted such that no conclusion can be made.
Sensitivity	Does the study have adequate sensitivity to detect an effect from exposure (if present)?	Sensitivity considers statistical power, exposure contrast, latency, and relevance of the exposure metric.	***Minimal concerns*** The study had an adequate number of exposed animals, with substantial exposure (level, duration, or range) and with adequate duration of follow-up for latency status. ***Some concerns*** The study has adequate sensitivity for some but not all elements. ***Critical or major concerns*** The study was modest or small, with few exposed animals, and/or the exposure range was minimal.

2.4.2. Overall Study Informativeness

The overall informativeness of a study considers both bias (e.g., systematic flaws or limitations that may compromise the interpretation of the results), and study sensitivity. Studies having elements with major concerns may still be considered for cancer hazard assessment, but the findings should be interpreted with caution. It should also be noted that some concerns about a study element (such as inadequate observation and/or exposure period or statistical power) would decrease the study's sensitivity to detect an effect. If positive findings were described despite these limitations, these studies would inform a cancer hazard assessment. Studies with critical concerns about important issues are generally inadequate to inform the evaluation.

If a study has inadequate information for a reviewer to answer a specific question, the impact on overall study quality evaluation depends on the extent and importance of the missing information and is evaluated on a case-by-case basis.

Study informativeness-level judgment

- ***High*** (no/minor concerns about most potential biases, high or moderate sensitivity)
- ***Moderate*** (some concerns about most potential biases)
- ***Low*** (major concerns about several biases, sensitivity rating varies); depending on the direction and distortion of the potential biases, the study may still be informative for cancer hazard evaluation but should be viewed with caution
- ***Inadequate*** (critical concerns about any bias, sensitivity rating varies)

2.5. Evidence Evaluation and Integration

2.5.1. Interpretation of the Evidence from Individual Studies

For each study, we use a weight of evidence approach to determine whether a tumor site is treatment-related (e.g., biological significance), based on the considerations listed below. For each tumor site of interest, we make a judgment about the study conclusions, e.g., evidence, some evidence, equivocal, null, or unable to determine. Note, we may not be able to make a determination because of inadequate reporting.

- Metrics: tumor incidence, multiplicity, time to tumor, magnitude of the effect, dose response. Note that dose-response does not have to be monotonic. Some animal cancer studies include extra animals designated for sacrifice at times earlier than the termination of the study, often at 1 year. These animals should be pre-designated as separate from the main study and not be included in the calculations of tumor incidences in the main study.
- Statistical related factors: significance, dose-related trends. Note that pairwise and trend tests are not both required.
- Historical controls: Provide context for interpreting the results, primarily for rare tumors or spontaneous tumors with high variability. Historical controls

should be for the same species, sex, duration, etc. and from the same lab, source, and calendar period (e.g., within 2 to 3 years) as the study, as background tumor rates can vary.

- Biological related factors: non-neoplastic lesions, preneoplastic lesions at the tumor site of interest, survival.
- Tumor consideration: rarity, background rates, historical controls, malignant vs. benign, or malignant and benign combined.
- Study informativeness assessment and related considerations, potential and direction for bias, sensitivity, species, sex, strain.

External Validity

External validity addresses the extent to which conclusions from one study can be generalized to other situations (i.e., the relevance of experimental animal data to humans). Neoplasms observed in experimental animals are considered relevant to humans unless there is *compelling* evidence indicating that they occur by a mechanism that does not operate in humans. We considered the following in assessing the relevance of an experimental animal cancer study for evaluating the potential for human carcinogenicity:

- Relevance of the route of exposure
- Relevance of the species, sex, or animals' age
- Relevance of the mechanism of tumor formation (e.g., strong evidence that the mechanism does not occur in humans)

All exposure routes of studies identified to date are relevant for glyphosate exposure.

2.5.2. Evidence Integration Across Animal Cancer Studies

The final steps in evaluating evidence from experimental animal cancer studies are integrating the evidence for treatment-related tumors across studies, applying the RoC listing criteria, and reaching a level-of-evidence conclusion from studies in experimental animals. The conclusion will be based on integration of findings from experimental animal and veterinary epidemiology studies.

RoC listing criteria for evaluating carcinogenicity from studies in experimental animals[1]

Sufficient evidence of carcinogenicity from studies in experimental animals:
An increased incidence of malignant and/or a combination of malignant and benign tumors

- in multiple species, or
- at multiple tissue sites, or
- by multiple routes of exposure, or

[1] The steps related to reaching level-of-evidence conclusions will only be completed for the internal cancer hazard assessment, and will not be carried out for journal publications.

- to an unusual degree with regard to incidence, site, or type of tumor or age at onset.

The first step in evidence integration is to evaluate the evidence across studies in rodents and dogs for each cancer site of interest and for each exposure (glyphosate and GBH) based on the individual study evaluations discussed above.

For most databases, heterogeneity in findings is often explained by differences in experimental conditions (e.g., species, sex, strain, doses, duration, route). As mentioned above, the most informative studies (highest quality and sensitivity) are given the most weight. Moderate- and low-quality studies can also be used in the assessment, especially when it is unlikely that biases (moderate/major) in the studies would cause false-positive results. Replication of findings across several studies also increases confidence in treatment-related effects; however, studies done in the same species or sex are not necessarily replicates as study conditions can vary across calendar time and laboratory. We do not plan on conducting a meta-analysis.

The second step is to apply the RoC criteria to the treatment-related effect assessment[2]. In general, the RoC criteria for sufficient evidence of carcinogenicity from studies in experimental animals are fulfilled by (1) two studies (by different exposure routes or in different species) reporting positive findings of malignant or combined malignant and benign tumors or (2) one study reporting positive findings at multiple tissue sites. In addition, positive findings from one robust study can fulfill the criteria if the tumors are rare, have an early onset, or have a high incidence. The spectrum of neoplastic responses, from pre-neoplastic lesions and benign tumors to malignant neoplasms of a specific tumor type, is relevant for the evaluation of whether benign tumors observed at increased incidences are likely to progress to malignancy. Note that we are only making a draft conclusion about the level of evidence from studies in experimental animals and not a listing recommendation for the RoC.

2.6. Reporting of the Cancer Hazard Assessment

As mentioned in section 2.1, for each study, we collated all available study information into a single study document to evaluate the totality of available information of a given study. This compiled document will be used to extract data in a web-based software, Table builder. The database is organized by study methods, including study design (e.g., species, strain, route), exposure methods (e.g., agent, route, dosing regimens), outcome methods (e.g., tumor incidence, animal survival), information related to evaluating confounding (e.g., animal husbandry methods, chemical purity), and statistical analyses. Quality assurance of data extraction and database entry is done by a reviewer independent of the data extractor. Any discrepant entries are resolved through mutual discussion with reference to the original data source. The extracted data are presented as tables in the monograph and can be downloaded into Excel for additional analyses or visualization.

[2] The steps related to reaching level-of-evidence conclusions will only be completed for the internal cancer hazard assessment, and will not be carried out for journal publications.

The assessment will be written in narrative style and include an overview of the study characteristics, study informativeness, evidence evaluation, and evidence integration, including an assessment for each tumor type of interest, and sensitivity analyses by study informativeness. Study results will be presented in tables or graphs. The level of evidence for carcinogenicity from studies in experimental animals (sufficient, not sufficient) and rationales for the conclusions are presented in evidence-based tables.

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Appendix A. Roles and Responsibilities

A.1. Evaluation team:

Evaluation teams are composed of federal staff and contractor staff. Procedures are in place to avoid actual or perceived conflicts of interest. Members of the evaluation team have experience or training in conducting literature searches and/or evaluating occupational and environmental epidemiology studies.

A.1.1. NIEHS Core Project Leaders

Develops research concept, rationale, and framework; serves as a researcher

- Ruth Lunn, DrPH [overall project]
- Suril Mehta, DrPH [overall project]
- Sabah Quraishi, PhD [human cancer]
- Matthew Urich, PhD [animal cancer]

A.1.2. Team Members

Responsibilities	Member	Affiliation
Overall		
Project management	Whitney Arroyave, PhD	Inotiv
Develop search strategy, conduct literature searches, and manage literature	Rachael Kalsch, MLIS	Inotiv
Literature management, document preparation, visualization	Tracy Sauders, BS	Inotiv
Investigation, writing, review and editing	Bevin Blake*, PhD	NIEHS
Animal cancer assessment		
Researcher: toxicological expertise, study informativeness assessment, data extraction, writing, critical review	Ezazul Haque, PhD John Bucher, PhD Matthew Urich, PhD	NIEHS
	Danila Cuomo, PhD	Inotiv
Human Cancer Assessment		
Researcher: Epidemiological expertise, study informativeness assessment, data extraction, writing, critical review	Ruth Lunn, DrPH Suril Mehta, DrPH Sabah Quraishi, PhD	NIEHS
	Whitney Arroyave, PhD	Inotiv

*Joined the evaluation team after the protocol was finalized, however was involved in the assessment and report writing.

A.2. Technical Advisors and Reviewers

Expertise or Role	Member	Affiliation
Statistical analyses	Keith Shockley	NIEHS
Pathology	Sonika Patial, PhD, DVM	NIEHS
Protocol reviewer	Chad Blystone, PhD Kristen Ryan, PhD Amy Wang, PhD	NIEHS

Appendix B. Citation Database Search Terms

Pubmed

((((("glyphosate"[nm] OR "1071-83-6"[tw] OR "(Carboxymethylamino)methylphosphonic acid"[tw] OR "Carboxymethylaminomethanephosphinic acid"[tw] OR "C-K Yuyos FAV"[tw] OR "CP 67573"[tw] OR "Folusen"[tw] OR "Forsat"[tw] OR "Glialka"[tw] OR "Glifoglex"[tw] OR "Glifosan 747"[tw] OR "gliphosate"[tw] OR "Gliz"[tw] OR "Glyfos"[tw] OR "GlyGran"[tw] OR "Glyphodin A"[tw] OR "Glyphomax"[tw] OR "Glyphosate"[tw] OR "Glyphosphate"[tw] OR "Ground Bio"[tw] OR "Herbatop"[tw] OR "HM 2028"[tw] OR "Kickdown"[tw] OR "Lancer herbicide"[tw] OR "MON 2139"[tw] OR "MON 3539"[tw] OR "MON 6000"[tw] OR "N-(Phosphonomethyl)glycine"[tw] OR "N-(phosphonomethyl)-Glycine"[tw] OR "N-Phosphomethylglycine"[tw] OR "NPhosphonomethylglycine"[tw] OR "Phorsat"[tw] OR "Phosphonomethylglycine"[tw] OR "Phosphonomethyliminoacetic acid"[tw] OR "Pondmaster"[tw] OR "Rebel Garden"[tw] OR "Roundup Max"[tw] OR "Safal"[tw] OR "Scout herbicide"[tw] OR "Silglif"[tw] OR "yerbimat"[tw] OR "Roundup"[tw] OR "34494-03-6"[tw] OR "MON 0459"[tw] OR "40465-66-5"[tw] OR "MON 14420"[tw] OR "MON 8750"[tw] OR "Roundup Hi-Load"[tw] OR "Roundup PRODry"[tw] OR "70393-85-0"[tw] OR "MON 8000"[tw] OR "Monsanto 8000"[tw] OR "Polado"[tw] OR "Trisodium hydrogen bis(N-(phosphonatomethyl)aminoacetate"[tw] OR "39600-42-5"[tw] OR "Glyphosate potassium"[tw] OR "Glyphosate monopotassium salt"[tw] OR "Glyphosate potassium"[tw] OR "Glyphosate-potassium"[tw] OR "Monopotassium glyphosate"[tw] OR "Roundup Attack"[tw] OR "Roundup Energy"[tw] OR "Roundup Maxload"[tw] OR "Roundup Original Max"[tw] OR "Roundup Power Max"[tw] OR "Roundup Ultramax II"[tw] OR "Roundup Weathermax"[tw] OR "Touchdown Forte HiTech"[tw] OR "Transorb R"[tw] OR "Weathermax"[tw] OR "Zapp Qi"[tw] OR "70901-12-1"[tw] OR "Glyphosate-potassium"[tw] OR "Potassium glyphosate"[tw] OR "Potassium N-(phosphonomethyl)glycine"[tw] OR "Uragan Forte"[tw] OR "VisionMAX"[tw] OR "N-(phosphonomethyl)glycine potassium salt"[tw] OR "114370-14-8"[tw] OR "Glyphosate ammonium"[tw] OR "N-(phosphonomethyl)glycine ammonium salt"[tw] OR "69254-40-6"[tw] OR "Glyphosate-diammonium"[tw] OR "Diammonium N-(phosphonomethyl)glycine"[tw] OR "N-(phosphonomethyl)glycine diammonium salt"[tw])) OR (("glyphosate, isopropyl amine salt"[nm] OR "N-(phosphonomethyl)glycine trimethylsulfonium salt"[nm] OR "38641-94-0"[tw] OR "Glyphosate-isopropylammonium"[tw] OR "Glyphosate isopropylamine salt"[tw] OR "Azural AT"[tw] OR "CP 70139"[tw] OR "Fosulen"[tw] OR "Glifosato estrella"[tw] OR "Glycel"[tw] OR "Glycine, N-(phosphonomethyl)-, cmpd with 2- propanamine (1:1)"[tw] OR "Glyfos AU"[tw] OR "Glyfos BIO"[tw] OR "Glyphosate isopropylamine salt"[tw] OR "Glyphosate mono(isopropylamine) salt"[tw] OR "Glyphosateisopropylammonium"[tw] OR "Glyphosate-mono(isopropylammonium)"[tw] OR "Landmaster"[tw] OR "MON 139"[tw] OR "MON 39"[tw] OR "N-(Phosphonomethyl)glycine isopropylamine salt"[tw] OR "N-(Phosphonomethyl)glycine isopropylammonium salt"[tw] OR "N-(Phosphonomethyl)glycine monoisopropylamine salt"[tw] OR "Nitosorg"[tw] OR "Rondo"[tw] OR "Utal"[tw] OR "Utal (herbicide)"[tw] OR "Vision (herbicide)"[tw] OR "2- Propanamine, compd, with N-(phosphonomethyl)glycine (1:1)"[tw] OR "Glycine, N-

(phosphonomethyl)-, compd. with 2-propanamine (1:1)"[tw] OR "N-(Phosphonomethyl)glycine, compound with 2-propylamine (1:1)"[tw] OR "Isopropylamine glyphosate"[tw] OR "81591-81- 3"[tw] OR "Glyphosate-trimesium"[tw] OR "Glyphosphate-trimesium"[tw] OR "Avans 330"[tw] OR "Glyphosate mono(trimethylsulfonium) salt"[tw] OR "Glyphosate trimethylsulfonium salt"[tw] OR "Glyphosate-trimesium"[tw] OR "Medallon"[tw] OR "Ouragan"[tw] OR "R 50224"[tw] OR "SC 0224"[tw] OR "Sulfosate"[tw] OR "Sulphosate"[tw] OR "Touchdown herbicide"[tw] OR "Trimethylsulfonium carboxymethylamino-methylphosphonate"[tw] OR "Trimethylsulfonium glyphosate"[tw] OR "Glycine, N-(phosphonomethyl)-, ion(1-), trimethylsulfonium"[tw] OR "Sulfosate"[tw]))) OR (("Aminomethylphosphonic acid" OR (AMPA and metabolite)) OR (“N-acetyl AMPA”) OR ("N-acetyl glyphosate")))

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ts=((((("glyphosate" OR "1071-83-6" OR "(Carboxymethylamino)methylphosphonic acid" OR "Carboxymethylaminomethanephosphinic acid" OR "C-K Yuyos FAV" OR "CP 67573" OR "Folusen" OR "Forsat" OR "Glialka" OR "Glifoglex" OR "Glifosan 747" OR "gliphosate" OR "Gliz" OR "Glyfos" OR "GlyGran" OR "Glyphodin A" OR "Glyphomax" OR "Glyphosate" OR "Glyphosphate" OR "Ground Bio" OR "Herbatop" OR "HM 2028" OR "Kickdown" OR "Lancer herbicide" OR "MON 2139" OR "MON 3539" OR "MON 6000" OR "N-(Phosphonomethyl)glycine" OR "N-(phosphonomethyl)-Glycine" OR "N-Phosphomethylglycine" OR "NPhosphonomethylglycine" OR "Phorsat" OR "Phosphonomethylglycine" OR "Phosphonomethyliminoacetic acid" OR "Pondmaster" OR "Rebel Garden" OR "Roundup Max" OR "Safal" OR "Scout herbicide" OR "Silglif" OR "yerbimat" OR "Roundup" OR "34494-03-6" OR "MON 0459" OR "40465-66-5" OR "MON 14420" OR "MON 8750" OR "Roundup Hi-Load" OR "Roundup PRODry" OR "70393-85-0" OR "MON 8000" OR "Monsanto 8000" OR "Polado" OR "Trisodium hydrogen bis(N-(phosphonatomethyl)aminoacetate" OR "39600-42-5" OR "Glyphosate potassium" OR "Glyphosate monopotassium salt" OR "Glyphosate potassium" OR "Glyphosate-potassium" OR "Monopotassium glyphosate" OR "Roundup Attack" OR "Roundup Energy" OR "Roundup Maxload" OR "Roundup Original Max" OR "Roundup Power Max" OR "Roundup Ultramax II" OR "Roundup Weathermax" OR "Touchdown Forte HiTech" OR "Transorb R" OR "Weathermax" OR "Zapp Qi" OR "70901-12-1" OR "Glyphosate-potassium" OR "Potassium glyphosate" OR "Potassium N-(phosphonomethyl)glycine" OR "Uragan Forte" OR "VisionMAX" OR "N-(phosphonomethyl)glycine potassium salt" OR "114370-14-8" OR "Glyphosate ammonium" OR "N-(phosphonomethyl)glycine ammonium salt" OR "69254-40-6" OR "Glyphosate-diammonium" OR "Diammonium N-(phosphonomethyl)glycine" OR "N-(phosphonomethyl)glycine diammonium salt")) OR (("glyphosate, isopropyl amine salt" OR "N-(phosphonomethyl)glycine trimethylsulfonium salt" OR "38641-94-0" OR "Glyphosate-isopropylammonium" OR "Glyphosate isopropylamine salt" OR "Azural AT" OR "CP 70139" OR "Fosulen" OR "Glifosato estrella" OR "Glycel" OR "Glycine, N-(phosphonomethyl)-, cmpd with 2- propanamine (1:1)" OR "Glyfos AU" OR "Glyfos BIO" OR "Glyphosate isopropylamine salt" OR "Glyphosate mono(isopropylamine) salt" OR "Glyphosateisopropylammonium" OR "Glyphosate-mono(isopropylammonium)" OR "Landmaster" OR "MON 139" OR "MON 39" OR "N-(Phosphonomethyl)glycine isopropylamine salt" OR "N-(Phosphonomethyl)glycine isopropylammonium salt" OR

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