Glyphosate Scientific Review
Final Phase 2 Report

Prepared for:

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Executive Summary

This Glyphosate Scientific Review summarizes information on uses, usage quantities, human health impacts, environmental impacts, and alternatives of the herbicide, glyphosate. Eastern Research Group, Inc. (ERG) prepared this report under contract to the Massachusetts Department of Agricultural Resources (MDAR). This legislatively mandated report will be presented to the Massachusetts Glyphosate Commission and then submitted to the joint Committee of Environment, Natural Resources and Agriculture for consideration during MDAR's glyphosate review process. This executive summary highlights several of the review's key findings and directs readers to the corresponding report sections for more detailed information. Section 1.0 of this report has additional information on the project's scope and research methods. This Review is largely based on scientific publications and major assessments identified in a document search completed in January 2023. Additional scientific publications and major assessments issued since that cutoff date are not reflected in the contents of this report.

Background. Glyphosate is a synthetic, non-specific, systemic herbicide that controls many types of weeds and other vegetation by interfering with the shikimate pathway, which plants need to produce amino acids and to survive. Glyphosate is the active ingredient in many herbicide products, referred to in this report as glyphosate-based formulations (GBFs). GBFs contain a range of chemicals in addition to glyphosate. In 1974, EPA registered the first product that contained glyphosate. EPA is in the process of reregistering glyphosate, and that process is expected to continue until 2026. MDAR has registered more than 100 GBFs for use in Massachusetts, most of which are general use (as opposed to restricted use) pesticides.

Section 2.1 has additional background information on glyphosate and GBFs.

Uses and usage quantities. According to the U.S. Environmental Protection Agency (EPA), glyphosate is one of the most widely used herbicides in North America. It is used in agricultural, commercial, industrial, residential, and many other settings. No published estimates are available on the amount of glyphosate (or GBFs) used in Massachusetts. However, the available information suggests that non-agricultural glyphosate uses in Massachusetts are considerably higher than agricultural uses. The pesticide product labels have instructions for how the GBFs should be used, what uses to avoid, and how applicators can protect themselves from toxic chemical exposure.

Sections 2.2 through 2.4 have further information on glyphosate uses and usage quantities in Massachusetts.

Human health impacts. The primary mechanism of glyphosate's herbicidal action—killing weeds and other vegetation by interfering with the shikimate pathway—does not apply to mammals, because they do not have this pathway. However, this does not rule out possible impacts on other mechanistic pathways in mammals. After humans ingest glyphosate, whether in diet or water, glyphosate is primarily absorbed in the human gut. Glyphosate does not readily accumulate in human organs or tissues. Though a portion of absorbed glyphosate is metabolized in humans, most of the absorbed glyphosate is excreted unmetabolized in urine. The Centers for Disease Control and Prevention has recently reported detecting glyphosate in 81 percent of urine samples collected from a random subset of the U.S. population over the age of six—a finding that likely reflected recent dietary exposures.

In 2017, EPA completed a draft human health risk assessment of glyphosate. Among other key findings, EPA has reported that glyphosate presents "no risks of concern to human health from current uses"; "no indication that children are more sensitive to glyphosate"; "no indication that glyphosate is an endocrine disruptor"; and is "not likely to be carcinogenic to humans." Findings from other major assessments, including in draft products from an ongoing assessment being conducted in the European Union, reached similar conclusions.

Whether glyphosate causes cancer is one topic where the major scientific assessments have inconsistent findings. The International Agency for Research on Cancer (IARC) classified glyphosate as "probably carcinogenic to humans," while every other major assessment that ERG reviewed reported finding no evidence or insufficient evidence to make that connection. It appears that IARC and EPA reached different conclusions because they based their assessments on different research studies, because they weighted the studies that they selected differently, and because they applied different criteria when deciding which underlying data are relevant (e.g., IARC considered evidence for genotoxicity in non-mammalian species and EPA did not). EPA's finding generally aligns

with those issued by government agencies in other countries, and it was vetted by an independent peer review conducted by expert scientists.

This scientific review also considered peer-reviewed publications issued since EPA and other parties completed their most recent assessments. For most health endpoints considered (i.e., cancer, neurotoxic effects, endocrine effects, developmental effects, renal effects), ERG concluded that it is unlikely that the recent literature would alter findings in the various major agency assessments. For developmental effects, some recent research identified effects at considerably lower glyphosate exposure doses than reported in previous assessments. However, it is unclear if the underlying research protocols used would qualify for consideration in agency derivation of toxicity guidelines. The pending assessment being conducted in the European Union and the ongoing EPA assessment of glyphosate are expected to provide further context on this matter.

Section 2.5 has further information on the information that ERG reviewed when commenting on glyphosate's potential human health impacts.

Environmental impacts. Researchers and government agencies have investigated the extent to which applications of glyphosate and GBFs in aquatic and terrestrial settings affect a range of non-target organisms, including invertebrates, amphibians, fish, mammals, and plants. This review considered evidence summarized in EPA's 2015 preliminary ecological risk assessment, EPA's 2021 *Biological Evaluation for Glyphosate*, publications issued during the ongoing assessment conducted by the European Food Safety Authority, and the recent peer-reviewed literature.

These assessments consistently found that glyphosate is acutely toxic to nearly every plant species, except those genetically modified to tolerate glyphosate and those that have developed glyphosate resistance. EPA's assessment determined that glyphosate is "likely to adversely affect" several species through exposure to some combination of direct application, overspray, spray drift, or runoff. However, this "likely to adversely affect" designation is based on highly conservative analyses, and it does not mean that entire species are in jeopardy or that critical habitats are being adversely modified. Rather, this determination is primarily intended to identify the subset of issues to be further evaluated by EPA in consultation with other agencies (e.g., National Marine Fisheries Service, National Forest Service) for a Biological Opinion on whether potential effects to individuals might negatively impact populations or the entire species or adversely impact a critical impact and whether risk mitigation measures are warranted. More detailed information on potential impacts will become available in the forthcoming Biological Opinion, as well as in the final assessment that the European Food Safety Authority is expected to issue later in 2023.

Section 2.6 has further information on the information that was considered when commenting on glyphosate's potential environmental impacts.

Alternatives. Various weed and vegetation control strategies have been proposed and used as glyphosate alternatives. These alternatives are classified in this report as chemical methods, mechanical methods, physical methods, and biological methods. Many different factors must be considered when evaluating alternatives, including effectiveness at controlling the target organism (both over the near term and long term), potential environmental and human health impacts, ease of implementation, the number of applications required, public acceptance, applicable regulations and restrictions, and cost. This report considers the viability for using glyphosate alternatives as weed control for multiple specific applications. While alternatives may be effective for certain glyphosate uses, this research did not identify any other systemic, non-selective herbicides with comparable effectiveness to glyphosate across all applications.

Section 3.0 has further information on ERG's research on glyphosate alternatives.

1.0 Introduction

In 2021, the Massachusetts legislature enacted the Acts of 2021. Chapter 24 of this legislation established budgets for many state government activities, including the formation of a commission charged with conducting "a scientific review of the potential impacts of glyphosate and its most common alternative herbicides on the environment and public health" (Commonwealth of Massachusetts 2021).

The legislation further states that: "…the pesticide subcommittee established under section 3A of chapter 132B of the General Laws shall use said scientific review as part of an individual review conducted under 333 C.M.R. 8.03 to determine whether current uses of glyphosate pose unreasonable adverse effects to the environment, and whether current registered uses of glyphosate should be altered or suspended" (Commonwealth of Massachusetts 2021).

Pursuant to the Acts, the Glyphosate Commission was formed, and the Commission opted to use contractor support to conduct the glyphosate scientific review. The Massachusetts Department of Agricultural Resources (MDAR), on behalf of the Glyphosate Commission, issued a Request for Quotes to seek contractor support for this project. After an open bidding process, MDAR issued a contract to Eastern Research Group, Inc. (ERG) to conduct the scientific review of glyphosate and its alternatives. The review is to consider uses, restrictions, public health impacts, and environmental impacts of glyphosate. The results of the review will be presented to the Glyphosate Commission and then submitted to the joint Committee of Environment, Natural Resources and Agriculture.

MDAR split the glyphosate scientific review project into two phases. In Phase One, MDAR tasked ERG with identifying all resources to consider for the scientific review; ERG would then review and summarize those resources in Phase Two. ERG, with assistance from its subcontractor Tetra Tech, Inc., submitted the final Phase One report in December 2022 (ERG 2022). ERG, again with assistance from Tetra Tech, Inc., prepared this Phase Two report, which is organized into the following sections:

- The remainder of this section describes the project scope, information sources that the ERG team considered, public input opportunities, and this report's review process.
- Section 2.0 summarizes the ERG team's findings on glyphosate. The section provides background information on glyphosate, identifies glyphosate uses in Massachusetts, summarizes information on glyphosate usage quantities, and reviews evidence for human health and environmental impacts.
- Section 3.0 summarizes the ERG team's findings on glyphosate alternatives, considering chemical, mechanical, physical, and biological methods.
- Section 4.0 lists the references cited throughout this report.
- Section 5.0 lists the abbreviations used in this report.

1.1 Project Scope

The scope for the glyphosate scientific review project is documented in ERG's contract with MDAR, and the structure of this report reflects the scope: one section reviews uses, usage quantities, human health impacts, and environmental impacts of glyphosate; another section reviews information on selected glyphosate alternatives.

An important element of the scope was whether this scientific review should evaluate both glyphosate (and its salts) and glyphosate-based formulations (GBFs). This is an important distinction because glyphosate and its salts are the active ingredients with intended pesticidal action, and GBFs contain various other ingredients (e.g., carriers, solvents, surfactants) in addition to glyphosate. These other ingredients are typically added to enhance application and increase effectiveness; while they do not have intended pesticidal action, many of these other ingredients can be toxic and can impact human health and the environment. Although the identities of some non-glyphosate ingredients in GBFs are publicly known, many are not because manufacturers are not required to disclose them.

Use of additional ingredients is not unique to GBFs. Many non-glyphosate pesticide formulations also combine active ingredients with other ingredients to make the products perform as intended for the specific use scenarios listed on the product labels. All ingredients used in registered pesticides must be approved by the U.S.

Environmental Protection Agency (EPA). Section 2.1 provides further information on how EPA registers pesticides, considering both active ingredients and all other ingredients.

During Phase One, the Glyphosate Commission confirmed that this review is to focus on human health and environmental impacts of glyphosate, which may differ from the impacts of GBFs. Accordingly, this scientific review primarily focuses on glyphosate and its salts. This scientific review does not comprehensively assess all GBFs or the various non-glyphosate ingredients within GBFs; however, the toxicity of other ingredients is acknowledged, as appropriate, when interpreting the human health and environmental impacts documented in the peer-reviewed literature.

Section 2.1 provides further information on the GBFs that are registered for use in Massachusetts and their ingredients, to the extent their compositions are publicly available.

1.2 Publications and Information Resources Considered

The ERG team was charged with reviewing published information on glyphosate's human health and environmental impacts, primarily considering assessments issued by recognized authorities and peer-reviewed publications. All assessments identified in the Phase One report were evaluated for this review. The Phase One report also outlined the process that the ERG team would follow to identify relevant peer-reviewed publications issued in recent years, but it did not provide all associated details (e.g., the specific search strings, keywords, and search engines to use). Section 2.5.3 and Section 2.6.2 of this report describe the literature searches that the ERG team conducted to inform the evaluation of glyphosate-related human health impacts and environmental impacts, respectively. This literature searches were completed in January 2023. Additional scientific publications and major assessments issued since that cutoff date are generally not reflected in the contents of this report. The public comments listed in Appendix A identify examples of scientific publications and major assessments that were issued since this project's literature cutoff date passed.

The scope of work initially called for the scientific review to consider precedential judicial decisions related to glyphosate. During Phase One, ERG asked the Glyphosate Commission to clarify this requirement, because a stakeholder voiced concern about the reliability of scientific information presented in legal proceedings. During its meeting in September 2022, the Glyphosate Commission confirmed that ERG's review of judicial decisions should focus on identifying publicly available and peer-reviewed scientific information on glyphosate's human health and environmental impacts that were not identified through ERG's other research for this project. ERG was not tasked with reviewing the status of the thousands of ongoing legal proceedings pertaining to glyphosate. This report does, however, consider the ongoing legal proceedings regarding glyphosate's registration status in the United States, and Section 2.1 and Section 2.5.2 discuss those proceedings further.

Another source of information during Phase Two was virtual interviews conducted by ERG with representatives from various state agency programs and subcommittees. ERG's scope of work called for summarizing glyphosate uses, regulations, and other management requirements from other Northeast states. ERG attempted to schedule interviews with senior state officials involved with pesticide registration and oversight in Connecticut, Maine, New Hampshire, New York, Rhode Island, and Vermont. Five of the six interviews were conducted. ERG also conducted interviews with all members of the Massachusetts Pesticide Board Subcommittee to ask about various issues related to Phase Two (e.g., information resources, issues of concern, and expectations for the report). ERG interviewed all four Subcommittee members and a designee of the fifth. Information from these virtual interviews is summarized in the appropriate sections of this report, primarily in Sections 2.2 and 2.3.

1.3 Public Input

The public was provided multiple opportunities to provide input for this glyphosate scientific review.

During Phase One, the Glyphosate Commission held three virtual meetings during which the scope of Phase One was discussed. These meetings were open to the public and included time for the public to ask questions and make comments. Further, in June 2022, the public was invited to comment on the draft Phase One report. Those comments were made available to the Glyphosate Commission and were considered by ERG when finalizing the Phase One report.

During Phase Two, the public was invited to provide input to the ERG team on two occasions. First, in January 2023, the Glyphosate Commission website was updated with a request for public input on Phase Two, and an email was circulated to the Commission's listserv about this public input opportunity. The request directed people to submit input to a representative from the Massachusetts Department of Environmental Protection (MassDEP). Five individuals submitted comments that the MassDEP representative forwarded to ERG for consideration. Second, in April 2023, ERG emailed the same request for public input to most of the non-government organization stakeholders listed in Section 4.0 of the Phase One report; current email addresses could not be located for some of these individuals. More than a dozen of these stakeholders submitted information to ERG in response to this inquiry. ERG reviewed all information submitted by the public when preparing this Phase Two report.

1.4 Phase Two Report Review

ERG submitted a draft of this Phase Two report to the Glyphosate Commission, who then made it available for public comment. The public was given one month to submit comments on the draft report. Two parties submitted comments, which are included (without modification) in Appendix A of this report. The comments addressed many topics, including requests for corrections, descriptions of scientific information that became available after this project's literature review cutoff date, and requests that additional details be added on certain issues. Tasked with revising the report to address factual errors, ERG made two revisions to the report. These revisions addressed comments that identified specific statements in the draft report that were not factually correct.

2.0 Findings on Glyphosate

This section first provides background information on glyphosate (Section 2.1), its uses (Section 2.2), usage quantities (Section 2.3), and restrictions (Section 2.4). It then reviews the information that the ERG team compiled regarding glyphosate's human health impacts (Section 2.5) and environmental impacts (Section 2.6).

2.1 Background Information on Glyphosate

Glyphosate is a synthetic, non-selective, systemic herbicide. It will control many types of vegetation, including weeds, grasses, annuals, perennials, and woody plants; it is translocated throughout plants after absorption.

Glyphosate kills plants by interfering with the shikimate pathway, which plants use to produce the amino acids essential for growth and survival. Mammals do not have this pathway; therefore, glyphosate's mechanism of herbicidal toxicity does not apply directly to humans. However, the shikimate pathway is found in microorganisms, which researchers have noted when considering how glyphosate might affect the gut microbiome in mammals (ATSDR 2020).

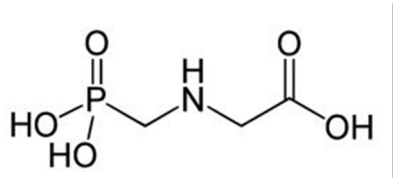
Though it is non-selective, glyphosate does not kill all plants. For example, some commonly produced crops (e.g., corn and soybeans) are available in genetically modified forms that are glyphosate tolerant. Farms that grow these crops can use glyphosate to control weeds and other plants without harming crop production. Development of these tolerant crops has contributed to a large increase in glyphosate usage in the United States (see Section 2.3). As another example, some weeds have become resistant to glyphosate (Baek et al. 2021).

Glyphosate is a solid at room temperature and is soluble in water. Figure 1 shows the chemical formula and molecular structure of glyphosate, which is found in many chemical and physical forms. Some herbicide products are formulated with glyphosate; others are formulated with various glyphosate salts, including the ammonium salt, diammonium salt, dimethyl ammonium salt, ethanolamine salt, isopropylamine salt, and potassium salt (EPA 2019a). The salts, once applied to the environment, are expected to dissociate rapidly and form glyphosate acid (EPA 2021).

FIGURE 1. GLYPHOSATE MOLECULAR STRUCTURE

Chemical formula: $C_3H_8NO_5P$

Molecular Structure:



An important consideration for this scientific review—and in most major scientific assessments for glyphosate (e.g., ATSDR 2020; IARC 2017)—is the distinction between "glyphosate technical" and "glyphosate-based formulations." For purposes of this review:

- Glyphosate technical refers to glyphosate and the salts noted above. These are the specific active ingredients with intended herbicidal action that product manufacturers registered with EPA.
- Glyphosate-based formulations (GBFs) refer to herbicide mixtures that contain glyphosate and its salts and other ingredients. The other ingredients serve various purposes. As one example, many GBFs contain surfactants that help ensure that glyphosate in mixtures spreads and remains on plant leaf targets, helping to enhance the effectiveness of the application. The composition of registered GBFs varies widely. Some contain less than 1 percent glyphosate (and therefore more than 99 percent other ingredients); others contain more than 90 percent glyphosate. While some non-glyphosate ingredients in GBFs are known (e.g., polyethoxylated tallow amine), many others are not.

EPA is the federal agency responsible for registering pesticides and regulating their use in the United States. In the registration process, the company that intends to produce a pesticide must first get approval from EPA. The company's application must specify the product's ingredients and their composition, information on the product's risks to human health and the environment, proposed labels, warnings, instructions for use, and other details. (Note, EPA is made aware of all ingredients in pesticides during the registration process, including ingredients that companies identify as confidential business information and do not specify on the product label.) Federal law also directs EPA to reregister pesticides considering the most up-to-date science.

As noted above, EPA evaluates the impacts of both the active ingredient in pesticides and the other ingredients, which are collectively referred to as inert ingredients. Only approved active and inert ingredients can be used in registered products. Despite what the name may imply, an inert ingredient does not mean that the ingredient is non-toxic. EPA makes safety determinations on pesticide products considering potential human health and environmental impacts from all ingredients (active and inert) in a formulation based on the proposed product uses. This review process applies to all pesticides that are registered with EPA.

In 1974, EPA registered the first herbicide product that contains glyphosate, and EPA completed a reregistration review in 1993 (EPA 2019a). In 2020, after conducting an additional review of glyphosate, EPA issued an interim decision on its review. Following legal challenges and a ruling issued by the U.S. Court of Appeals for the Ninth Circuit, EPA withdrew its interim decision for glyphosate, in part because the agency could not complete a court-ordered deadline for finalizing a portion of the registration review. EPA is in the process of revising its reregistration review, which the agency anticipates completing in 2026. Glyphosate-containing herbicides can continue to be used until then.

States also register pesticides. They may place additional limits on EPA-registered pesticides that are used within their jurisdictions, but states cannot register pesticides that have not been registered by EPA. MDAR is the Massachusetts agency that registers pesticides for use in the state, and details on MDAR-registered pesticides can be found on the Massachusetts Pesticide Product Registration Information website (Kelly Solutions 2023); however, just because a pesticide is registered for use in Massachusetts does not mean it is actually used. In May

2023, ERG queried the database for details on all Massachusetts-registered herbicides that contain the text "glyphosate" in its list of active ingredients. This query found records pertaining to 144 unique EPA registration numbers. These products' active ingredient concentrations, whether for glyphosate or one of its salts, ranged from 0.14 percent to 95.2 percent. Some of these products contained multiple active ingredients.

The Kelly Solutions database includes additional information on glyphosate-containing products registered in Massachusetts. A query on "pests controlled by this product" found a wide range of results. Consistent with the fact that glyphosate is non-selective, the majority of products list more than 100 pests that they control; examples include various grasses, weeds, poison ivy, and many others. A query on "sites to which this product may be applied" also yielded varying results. Roughly half of the products reviewed identified more than 100 sites to which the product may be applied; however, a few dozen products had fewer than ten sites listed, and this typically applied for products intended for agricultural use (e.g., application at sites that grow corn, soybeans, cotton, and sorghum) and for products limited to application on residential soils. Overall, this research noted a broad range of herbicide products that contain glyphosate and its salts, which are used for a wide range of pests and applied at many different types of sites.

The Kelly Solutions database includes links to the EPA-accepted labels for the various glyphosate-containing products registered for use in Massachusetts. These labels have information on application methods and rates, formulation details, approaches to minimizing spray drift, precautionary statements, steps to prevent resistance, and other topics. Glyphosate products are applied to target areas using a variety of mechanical devices, including hand-held or backpack sprayers, and other methods. The most appropriate application method depends on the size of the target area, the density of plant pests, concerns about impacts to surrounding areas, and other factors. The EPA-accepted labels provide further details on application methods for individual products. In most cases, labels warn users not to apply glyphosate-containing herbicides directly to water and outline steps users should take to prevent contamination of water resources; however, some glyphosate-containing herbicides can be used to control emergent aquatic weeds in certain circumstances.

Multiple companies, including Bayer Crop Science LP (Bayer), are registrants of glyphosate-containing herbicides that are registered in Massachusetts. Bayer manufactures the Roundup® product line and various other herbicides. In 2021, Bayer issued a press release indicating that, to mitigate litigation risks, it plans to replace glyphosate in "lawn and garden market" herbicides with an alternative active ingredient by 2023; the press release also noted that this decision was "not due to safety concerns" (Bayer 2021). Bayer indicated that it intends to continue to make glyphosate-containing herbicides available for agricultural and certain other uses (Bayer 2021). ERG notes these observations because, if Bayer implements its decision, the profile of glyphosate-containing herbicides used in the Commonwealth will change, although several companies other than Bayer currently manufacture glyphosate and glyphosate-containing herbicides.

2.2 Glyphosate Uses in Massachusetts

In the Phase One report, ERG identified the following categories of glyphosate uses in Massachusetts:

- Weed control for row crops (e.g., corn, soybeans, alfalfa)
- Weed control in orchards (e.g., apples)
- Weed control at nurseries
- Control of problematic plants (e.g., dodder, dewberries) in cranberry farming (UMass 2008)
- Control of nuisance plants in and along transportation rights of way (e.g., highways, railways)
- Residential and commercial landscape management to control weeds and unwanted plants
- Aquatic weed control as a restricted-use herbicide in MassDEP-permitted applications
- Habitat management for wildlife and unique ecosystems
- Control of invasive plants

ERG researched additional glyphosate uses, based on public input received in Phase One and Phase Two. For example, a comment on the Phase One report stated that the Massachusetts Department of Conservation and Recreation (DCR) uses glyphosate "pre- and post-harvest to kill native vegetation they deem unnecessary, despite the multitude of safe and effective alternatives." ERG contacted DCR to confirm the Department's current glyphosate uses and learned that the Department considers many factors when selecting herbicides for a given

application, and GBFs are typically not used when safer alternatives are available that can achieve the desired level of effectiveness. No other Phase One comments identified additional glyphosate uses in Massachusetts.

In Phase Two, the request for public input asked the following question: "Section 2.2 of the Phase One report lists categories of glyphosate uses that the Phase Two report will consider. What other glyphosate usage categories are relevant in Massachusetts and should be added to this list?" Several responses addressed this question and provided the following observations, some of which are related to the list provided above:

- Public utilities use glyphosate to manage vegetation along rights-of-way, reportedly including near areas where the groundwater is used as a drinking water source.
- Glyphosate (and other chemical herbicides) are used at schools and childcare centers.
- Commercial landscapers have many specific uses of glyphosate. These include control of weeds and plants that are poisonous or noxious, such as poison ivy, certain sumac plants, poison hemlock, and giant hogweed; spot treatment for grassy and broadleaf weeds that are not susceptible to selective herbicides; "blanket treatment" used before total renovation of existing stands or turfgrass; crack and crevice weed control in sidewalks, parking lots, and similar sites; and control or suppression of certain invasive species for which glyphosate is the only effective product.
- Glyphosate use for habitat management includes restoring the health and diversity of native, natural communities of Massachusetts.

Finally, during interviews with representatives of two other Northeast states' agriculture departments, ERG was informed of the use of glyphosate to remove cover crops in no-till farming applications and in "chemical mowing"; however, the extent to which this practice has been adopted in Massachusetts is not known. ERG's research also identified other glyphosate uses that may not be relevant to Massachusetts (e.g., use of glyphosate as a desiccant just prior to harvest of certain grains and other products).

2.3 Glyphosate Usage Quantities

ERG was charged with summarizing glyphosate usage quantities specific to Massachusetts. This section presents the information that ERG compiled for Massachusetts, along with glyphosate usage data for the entire United States for further perspective.

In 2021, EPA's biological evaluation of glyphosate notes that glyphosate "is one of the most widely used herbicides in North America" and provides usage statistics at the national level (EPA 2021). EPA estimated that nationwide *agricultural uses* of glyphosate active ingredients was 280 million pounds per year, based on underlying data from 2013 to 2018; agricultural applications were greatest in the Midwest, and applications to soybeans, corn, and cotton accounted for the greatest proportion of acreage treated (EPA 2021). EPA estimated that nationwide *non-agricultural uses* of glyphosate active ingredients were over 21 million pounds—more than an order of magnitude lower than the agricultural uses. Consumer applications in residential settings accounted for approximately one-fourth of the nationwide total non-agricultural uses; other large contributors to non-agricultural uses were applications to or near roadways and applications for forestry (EPA 2021). The national data are provided here for context only. Their representativeness to Massachusetts is limited, due to significant differences in agricultural practices across the United States—a point emphasized later in this section. The national data, however, reveal two trends of relevance: usage of glyphosate has increased dramatically over the last 50 years, and residential uses account for a considerable portion of non-agricultural uses.

ERG searched for estimates of glyphosate usage quantities for Massachusetts but was unable to find one that covers all uses. This is due to two reasons: One is because no party has tracked and published the amounts of glyphosate (e.g., as Roundup) sold in Massachusetts retail outlets for consumer uses; the other reason is because, as discussed during Glyphosate Commission meetings in 2022, annual usage reports that licensed applicators must submit to MDAR are only available in paper form and must be reviewed individually to estimate statewide usages, and this project's budget could not accommodate the estimated costs for manually reviewing the hard copy reports. ERG also considered all public input submissions received during Phase Two, but none offered glyphosate usage data sources beyond the ones that ERG considered below.

Despite these limitations, some data are available that allowed ERG to estimate glyphosate usage quantities in Massachusetts for selected categories of uses:

- *Agricultural glyphosate usage in Massachusetts.* Researchers from the U.S. Geological Survey (USGS) annually publish data on pesticide usage in the United States agriculture sector. The most recent data, from 2019, are aggregated by active ingredient, location, and crop (Wieben 2021). The data indicate that Massachusetts agricultural usage of glyphosate active ingredient in 2019 was 13,435 pounds. For a sense of scale, the state with the largest agriculture usage in 2019 was Iowa, with 21,560,000 pounds of active ingredient used (Wieben 2021). Across the contiguous United States, only Rhode Island and New Hampshire had lower agricultural glyphosate active ingredient usage than Massachusetts in 2019. The relatively low usage in Massachusetts reflects the smaller size of the state and its smaller proportion of land dedicated to agriculture uses (USDA 2023). The USGS data indicate the following breakdown of glyphosate usage quantities in 2019 for row crops in Massachusetts: 11,838 pounds for corn; 1,144 pounds for fruits and vegetables; 172 pounds for soybeans; 169 pounds for orchards; and 112 pounds for alfalfa (Wieben 2021).
- *Non-agricultural glyphosate usage in Massachusetts.* ERG did not identify any references that document glyphosate usage for the range of non-agricultural uses in Massachusetts; however, EPA's 2021 *Biological Evaluation for Glyphosate* presents non-agricultural usage data by region within the coterminous United States (EPA 2021). Table 1 summarizes EPA's data for the Northeast region, which includes Connecticut, Maine, Massachusetts, New Hampshire, New Jersey, New York, Rhode Island, and Vermont. These data indicate that the largest non-agricultural usage of glyphosate in the Northeast is consumer applications and applications to roadside rights of way. The various other non-agricultural applications combined account for 40 percent of non-agricultural glyphosate usage.

TABLE 1. ESTIMATED ANNUAL NON-AGRICULTURAL GLYPHOSATE ACTIVE INGREDIENT USAGE IN THE NORTHEAST

Non-Agricultural Usage Category	Annual Active Ingredient Applied (lb)	Percent of Total for Northeast
Aquatic uses	50,000	2%
Rights of way – roadways	600,000	25%
Rights of way – railways	20,000	1%
Rights of way – utility and pipeline	100,000	4%
Forestry	70,000	3%
Agriculture turf (e.g., grass or sod production)	1,000	0%
Applied by landscape contractors	10,000	0%
Applied by lawn care operators	200,000	8%
Institutional turf facilities (e.g., schools, parks)	200,000	8%
Golf courses	20,000	1%
Nursery and greenhouse ornamentals	300,000	12%
Applied by consumers	860,000 *	35%
Total	2,431,000	100%

Source: EPA 2021. Appendix 1-4, Table 3.

Notes: Data for individual categories were reported in publications from 2013 through 2016.
All percentages are rounded to the nearest whole number.
* For glyphosate quantities applied by consumers, the EPA report only presented a national estimate (5,000,000 pounds). ERG estimated the Northeast quantity from this national data point by assuming that the amounts applied by consumers is proportional to the population. For this estimate, ERG used 2022 population data reported by the U.S. census (US Census 2023), which indicated that the population of the eight Northeast states considered in EPA's data account for 17.3 percent of the population within the coterminous United States. The estimated "applied by consumers" quantity reported in the table is uncertain: it includes the uncertainty associated with the nationwide consumer usage quantity and the uncertainty associated with the assumption that the per capita usage of glyphosate for consumer applications does not vary regionally. Actual quantities applied by consumers in the Northeast might be higher or lower than the value shown.

ERG considered the relative amounts of agricultural and non-agricultural glyphosate usage in Massachusetts. To do so, ERG first estimated the non-agricultural usage in the state based on the regional data, assuming the uses are proportional to population. This assumption is considered a rough approximation due to underlying uncertainties (e.g., some non-agricultural usage categories, like forestry, might be inversely proportional to population). With Massachusetts accounting for 16.2 percent of the population of the eight Northeast states considered in EPA's usage estimates (US Census 2023), the estimated annual non-agricultural glyphosate active ingredient usage in Massachusetts is 395,000 pounds—approximately 30 times higher than the reported agricultural glyphosate usage. While this multiplier has uncertainties associated with the population-based assumptions used in the derivation, this analysis shows that glyphosate active ingredient usage in Massachusetts for non-agricultural purposes is likely considerably higher than that for agricultural purposes, even though the national trend was the opposite.

For further context on glyphosate usage potentially relevant to Massachusetts, ERG considered input received on the topic from other Northeast states during the interviews conducted in Phase Two. These interviews were with senior officials from state agencies responsible for registering pesticides. Among the five states considered, three publish annual pesticide usage reports summarizing quantities of pesticides used by licensed applicators. These reports present numerous tabulations, including one for annual usage quantities for different active ingredients (including glyphosate and its salts). However, none of the five states interviewed tracks the amounts of glyphosate used in consumer applications. Therefore, ERG's interviews with other Northeast states do not provide quantitative insights on glyphosate usage that would allow for better estimation of usage quantities in Massachusetts.

2.4 Glyphosate Restrictions and Requirements to Minimize Impacts

When registering pesticides, EPA classifies them as either "restricted use" or "general use." Restricted use pesticides cannot be sold to the public and may only be sold to and used by appropriately licensed applicators. General use pesticides, on the other hand, may be sold to anyone and (with few exceptions) used by anyone. Although glyphosate is an active ingredient in some restricted use pesticides, nearly every glyphosate-containing pesticide registered by EPA is for general use. All EPA-registered pesticides (including general use pesticides) have labels with requirements that users must follow, and state and federal agencies have the authority to enforce label requirements. States also have the authority to restrict the use of general use pesticides in their jurisdictions. ERG was tasked with documenting information on glyphosate restrictions and requirements to minimize impacts.

With respect to restrictions, ERG considered the extent to which other Northeast states have restricted the use of glyphosate-containing pesticides, based on the interviews conducted with representatives from five states. None of the states interviewed have designated glyphosate-based pesticides as restricted use. One state (New York) passed Assembly Bill A732B that prohibited application of glyphosate-based pesticides on state property, and subsequent regulation (6 NYCRR Part 325) has allowed for exemptions under certain circumstances and when no viable alternatives are available. In other Northeast states, bills proposing statewide glyphosate bans have been introduced into legislatures (e.g., Connecticut and Vermont), but the proposed legislation did not pass. The officials interviewed by ERG also referred to state laws and regulations that prohibit all pesticide use in certain circumstances (e.g., a ban on chemical pesticide use at K-8 schools and daycare centers in Connecticut), but these requirements do not apply uniquely to glyphosate.

During the interviews, ERG also sought information on the extent to which cities and towns in the Northeast have prohibited glyphosate uses. The interviewees explained that municipalities cannot preempt state authority for regulating pesticides and therefore cannot ban all uses within their jurisdictions; however, municipalities can enact policies and ordinances limiting their own use of glyphosate (e.g., in public works departments) or prohibiting glyphosate use on city-owned property. Numerous municipalities in the Northeast have indeed passed such ordinances; some apply to all chemical pesticides and others apply specifically to glyphosate.

Requirements to minimize impacts from glyphosate-based herbicides are noted on their respective EPA-accepted labels. Common label requirements for glyphosate and its salts are to avoid spray drift, to not allow people or pets to enter recently treated areas until the application has dried, and to not apply directly to water; however, the specific requirements vary from one registered pesticide to the next.

2.5 Human Health Effects of Glyphosate

This section summarizes the current state of knowledge on the human health effects of glyphosate based on results of major assessments from authoritative bodies and the most recent evidence from toxicological, epidemiological, and case reports published in the peer-reviewed literature. The section highlights areas of consensus among the available information. It also describes inconsistencies in the published literature and, where possible, attempts to explain why they occurred.

2.5.1 General Considerations for the Scientific Review of Human Health Effects

This section summarizes both cancer and non-cancer health impacts associated with glyphosate exposure. In reviewing the human health effects of glyphosate and evaluating the differences among study approaches and results, this section considers the following:

Differences between glyphosate technical and glyphosate-based formulations. This review focuses on evaluating the toxicity of the active ingredient: glyphosate and its salts (collectively referred to here as glyphosate technical). However, GBFs include additional ingredients, some of which are known to be toxic; and the identities of other ingredients are not always disclosed on product labels, because manufacturers can claim them as confidential business information. (However, identities of all ingredients are made available to EPA during the pesticide registration process.) While some studies report adverse health effects for exposures to GBFs, the presence of multiple toxic ingredients in these formulations complicates efforts to attribute adverse human health effects specifically to glyphosate. All summaries in this section acknowledge whether underlying studies examined toxicity of glyphosate technical or GBFs. Note further that studies of GBFs often consider concentrated formulations, which have limited relevance to actual exposures because pesticide applicators often use dilute GBF spray solutions. The concentrated formulations are relevant to occupational exposure scenarios, particularly for workers who handle and mix concentrated products. However, product labels have specific requirements for minimizing exposures (and potential health risks) associated with handling concentrated GBFs.

Metabolites of glyphosate. Most glyphosate that humans ingest is excreted as glyphosate and not metabolized (ATSDR 2020); however, the glyphosate that is metabolized in the human body primarily forms aminomethylphosphonic acid (AMPA). Therefore, this section presents information both on glyphosate toxicity and AMPA toxicity, given that AMPA forms in the human body after glyphosate exposure.

Effects levels. The scientific literature often reports effects levels associated with toxicology studies. Two commonly reported effects levels are the no observed adverse effect levels (NOAEL) and the lowest observed adverse effect level (LOAEL). These are typically reported in studies that expose laboratory animals to multiple doses of a toxic substance, in which case the NOAEL is the highest exposure dose at which no toxic effects were observed, and the LOAEL is the lowest exposure dose at which toxic effects were observed. Government agencies use these effects levels when deriving toxicity screening criteria, typically by applying safety factors or uncertainty factors to ensure the derived criteria are sufficiently health protective. This study reports NOAELs and LOAELs, as appropriate, because they are useful when comparing results across studies.

Study type and quality. A comprehensive understanding of glyphosate's human health effects requires integrating evidence from various types of studies, accounting for the studies' strengths, limitations, and different designs. While epidemiological studies directly investigate human populations, *in vivo* and *in vitro* studies provide complementary information on dose-response relationships, toxicokinetics, and mechanisms of action. Described below are the different types of studies reviewed and the factors considered when evaluating study quality.

- **Epidemiological studies.** Epidemiological studies assess human populations and real-world exposure scenarios and therefore are an important consideration in this scientific review. These studies have many different designs. Some of the most common are cohort, case-control, and cross-sectional designs. Well-designed and executed prospective cohort studies and case-control studies, particularly those with robust exposure characterization, can identify associations between environmental exposures and adverse health effects. Cross-sectional studies typically investigate health outcomes at a single point in time and are more prone to bias. No matter the study design, accurate and reliable exposure measures are critical for characterizing the relationship between glyphosate and health outcomes. Some studies use direct measures of exposure (e.g., measured glyphosate concentrations in drinking water) while other studies

use surrogate exposure metrics (e.g., number of years using GBFs). When summarizing the recent literature, this review describes how studies characterized exposures (if at all) and identifies associated uncertainties. Another issue to consider when interpreting epidemiological studies is confounding factors, which can cause studies to overstate or understate the relationship between exposures and adverse health effects. Bias to the null hypothesis of no effect due to exposure misclassification and limited statistical power to detect effects are additional limitations. This review describes the extent to which epidemiological studies accounted and controlled for confounding factors. It should be noted that virtually every epidemiological study considered for this review investigated exposure to GBFs and not technical glyphosate alone.

- ***In vivo* studies.** Most toxicity studies summarized here are *in vivo* animal studies, in which living animals, typically genetically similar, are dosed with a toxicant under a controlled laboratory setting and monitored for adverse effects. *In vivo* studies allow for the examination of whole-organism responses to specific glyphosate exposures. For this section, only mammalian studies were considered when evaluating evidence for human health impacts. (See Section 2.6 for glyphosate toxicity information on non-mammalian species.) In evaluating the quality of an *in vivo* study, expert judgment was used to consider study design, appropriateness of an animal model for the endpoint of interest, accurate exposure and outcome assessments, data analysis, and reproducibility. In many *in vivo* animal studies, the doses used are considerably higher than human exposure levels under typical environmental conditions. Therefore, adverse effects noted in the animal studies may not necessarily occur in humans exposed at lower levels.
- ***In vitro* studies.** *In vitro* studies examine how cells and tissues in a laboratory setting respond to exposures to toxic substances. These studies provide insights into the effects of a substance at the cellular or molecular level and on mechanisms of toxicity; however, because they focus on cells and tissues and not entire organisms (whether humans or animals), *in vitro* studies do not directly characterize whether health effects occur following exposures. As a result, this review briefly summarizes findings from *in vitro* studies considering factors such as appropriateness of the cell model selection, dose selection, and relevance to human health endpoints.
- **Review articles, meta-analyses, and pooled analyses.** In addition to considering individual toxicological and epidemiological studies, the literature review below summarizes results from review articles, meta-analyses, and pooled analyses. Review articles synthesize evidence from multiple individual studies and tend to provide a broader understanding of a given issue. Meta-analyses or pooled analyses quantitatively combine or reanalyze the data from multiple studies. This report considers conclusions from high-quality review articles, meta-analyses, and pooled analyses because they reflect research across multiple disparate studies. Several factors contribute to the quality of these types of articles, including clear research questions and eligibility criteria, rigorous evaluation of the methodological quality of the included studies, appropriate statistical methods, and sensitivity analyses that exclude studies with high risk of bias.
- **Case reports.** The peer-reviewed literature also documents case reports of adverse health effects following individuals' accidental or intentional exposures to glyphosate or GBFs. These reports typically describe very high exposure incidents at doses considerably greater than those experienced by the general population. Nonetheless, case reports provide direct evidence of human health effects; therefore, case reports are also summarized below.

2.5.2 Assessments Issued by Government Agencies and International Bodies

Numerous national and international bodies have evaluated the health effects of glyphosate in scientific assessment reports. These assessments varied in their scope and objectives. For example:

- Some assessments can be classified as hazard characterizations, which involve determining if a chemical might cause a specific health effect, including at very high doses. Others were risk assessments, which examine risks for developing adverse health effects under specific exposure scenarios.
- Some assessments focused exclusively on cancer, while others considered a range of non-cancer and cancer health outcomes.

- Each assessment was based on published (and sometimes unpublished) scientific studies that were available at a given point in time. The assessments used different literature search strategies, cutoff dates, and inclusion and exclusion criteria.

These and other differences can contribute to the assessments reaching different conclusions.

Table 2 describes each agency assessment considered in this review. The table includes brief statements that summarize the assessments' overall findings on human health effects. Additional background information and context for these assessments is described below. Further details on each assessment's conclusions related to specific health effects are provided in Sections 2.5.4 to 2.5.10.

U.S., European, and other international regulatory agencies have all approved the use of glyphosate-based pesticides in their jurisdictions. The underlying risk assessments considered different exposure scenarios: some evaluated health risks associated with using GBFs according to label instructions, others considered health risks associated with glyphosate residues on food items, and so on. For example, in the United States, EPA regulates the safety of pesticides and recently concluded that regarding glyphosate, "there are no dietary risks of concern for any segment of the population," and "there are no residential, non-occupational bystander, aggregate, or occupational risks of concern" (EPA 2020a). EPA's findings are based on exposure scenarios that assume glyphosate-based pesticides are used according to label instructions. Furthermore, EPA's assessment is largely based on evidence for glyphosate technical, not for GBFs.

In the European Union (EU), the European Food Safety Authority (EFSA) and the European Chemicals Agency (ECHA) regulate the use of pesticides. Generally, ECHA conducts a hazard assessment, and EFSA uses ECHA's hazard classification to conduct a risk assessment considering general population exposure from food residues. ECHA's most recent hazard assessment concluded that "classifying glyphosate as a carcinogenic, mutagenic or reprotoxic substance is not justified" (ECHA 2022). EFSA's reevaluation of human health risk was still ongoing when this report was first drafted in late 2023.

A few other regulatory bodies outside the U.S. and Europe have conducted their own health assessments of glyphosate. This review considered assessments by the Food Safety Commission of Japan (FSCJ), the Australian Pesticide and Veterinary Medicine Authority (APVMA), and the Pest Management Regulatory Agency (PMRA) of Health Canada.

Table 2 also describes health assessments from non-regulatory agencies, including a cancer hazard assessment conducted by the International Agency for Research on Cancer (IARC). IARC is an intergovernmental agency that is part of the World Health Organization (WHO). In 2015, IARC completed an assessment evaluating carcinogenicity for five pesticides and herbicides, including glyphosate. IARC's monograph concludes that glyphosate is "probably carcinogenic to humans." IARC's work was completed in 2015, and the version of the monograph posted to IARC's website is dated 2017 (IARC 2017). IARC's cancer classification was considered in assessments conducted by other agencies. For example, the California Office of Environmental Health Hazard Assessment (OEHHA) listed glyphosate as a chemical "known to the state to cause cancer" under California's Proposition 65, primarily based on IARC's classification. Section 2.5.4 provides further context on why IARC and other agencies reached different conclusions on glyphosate's carcinogenicity.

Health assessments from the following non-regulatory agencies are also summarized in this section:

- The Food and Agriculture Organization (FAO) of the United Nations and the Core Assessment Group on Pesticide Residues of the WHO convened a joint panel to evaluate the human health risks of consuming food products that contain pesticide residues.
- The Agency for Toxic Substances and Disease Registry (ATSDR) evaluates the health risks associated with hazardous substances in the U.S.
- The U.S. Forest Service (USFS) assesses the potential health effects of pesticides used in major forest programs and activities.

In addition to the above agency assessments, this scientific review considered two major long-term government-funded studies:

- The National Toxicology Program (NTP) falls within the U.S. Department of Health and Human Services and issues cancer classifications for selected hazardous substances. Although NTP has not yet classified glyphosate for carcinogenicity, NTP has published results from rodent tests and *in vitro* genotoxicity tests on glyphosate and GBFs.
- The Agricultural Health Study (AHS) is funded by the National Cancer Institute and the National Institute of Environmental Health Sciences (NIEHS) and has included collaboration from EPA and the National Institute for Occupational Safety and Health (NIOSH). The AHS is an ongoing prospective cohort study that examines adverse health effects among pesticide applicators and their spouses. The AHS is one of the largest pesticide studies of farmers and their family members in the world. Although the study is not specific to glyphosate, the investigators have published journal articles on relationships between non-cancer and cancer outcomes and glyphosate usage.

Results from these two ongoing studies have been included in agency assessments, and this review summarizes the studies' most recent results.

When reviewing the evidence for glyphosate-related adverse human health effects, Sections 2.5.4 to 2.5.10 start by summarizing findings from the major assessments and government-funded studies listed above and described in Table 2.

TABLE 2. HUMAN HEALTH ASSESSMENTS PUBLISHED BY GOVERNMENT AGENCIES AND INTERNATIONAL BODIES

Agency/Study	Background and Determination
U.S. Environmental Protection Agency (EPA)	**Background:** EPA issues pesticide registration decisions on the basis of assessed risk associated with proposed use patterns; and EPA has conducted multiple assessments of the health effects of glyphosate. The human health evaluation in EPA's latest interim registration review decision for glyphosate (ID) was finalized in January 2020 (EPA 2020a). In July 2022, the United States Court of Appeals for the Ninth Circuit issued a ruling that, among other things, "vacated and remanded the human health portion" of EPA's ID (EPA 2022b). An EPA press release has since indicated that, despite this ruling, its "underlying scientific findings regarding glyphosate...remain the same" (EPA 2022b). Therefore, for this scientific review, ERG reviewed the draft human health risk assessment and accompanying documents (EPA 2017b; 2017c; 2017d; 2017e; 2018). EPA's primary literature searches for the *Draft Human Health Risk Assessment* were completed on October 5, 2015. An additional search that focused on characterizing the carcinogenic potential of glyphosate was conducted on May 6, 2016. EPA also considered articles submitted to the agency as part of the public comment process for the ID. The final risk assessment included articles published as recently as 2019.
	Determination: EPA's ID did not identify any cancer or non-cancer human health risk associated with registered uses of glyphosate; and this finding considered exposure to glyphosate and toxic effects of its metabolites. EPA specifically found: "…no dietary risks of concern for any segment of the population, even with the most conservative assumptions applied in its assessments (e.g., tolerance-level residues, direct application to water, and 100 percent crop treated). The agency also concluded that there are no residential, non-occupational bystander, aggregate, or occupational risks of concern" (EPA 2020a).
Agency for Toxic Substances and Disease Registry (ATSDR)	**Background**: ATSDR develops toxicological profiles for hazardous substances found at Superfund sites. In ATSDR's 2020 *Toxicological Profile for Glyphosate*, ATSDR reviewed the available scientific literature to assess the potential human health hazards associated with glyphosate exposure (ATSDR 2020). The profile was based on an initial literature search completed in September 2017 and a supplemental search through October 2019.
	Determination: The toxicological profile separately reviews evidence for glyphosate's non-cancer and cancer effects. For non-cancer, ATSDR notes that a range of effects have been observed in laboratory animals following oral exposure, with gastrointestinal disturbances and salivary gland effects being the most sensitive; effects to the liver, eyes, kidneys, body weight, and other organ systems were also noted. ATSDR developed acute- and chronic-

Agency/Study	Background and Determination
	duration oral Minimum Risk Levels (MRLs) for glyphosate technical based on non-cancer gastrointestinal effects in animal studies. The profile reviews literature on glyphosate and cancer outcomes, but ATSDR does not classify carcinogenicity in the way that other authorities (e.g., EPA, IARC, NTP) do.
U.S. Forest Service (USFS)	**Background**: USFS has a mission to "sustain the health, diversity, and productivity of the Nation's forests and grasslands." In support of that mission, USFS has evaluated the toxicity of various herbicides. A 2011 report presents a human health and ecological risk assessment of glyphosate (USFS 2011).
	Determination: USFS reported separate human health findings for technical glyphosate and GBFs. USFS determined that the "preponderance of the available data…clearly indicates that the mammalian toxicity of glyphosate [technical] is low, and very few specific hazards can be identified." In contrast, USFS describes the hazard of GBFs as "much less clear." USFS further states that the toxicity of "surfactants [in GBFs] is of equal or greater concern to the risk assessment than is the toxicity of technical grade glyphosate." For cancer effects, USFS reports that an earlier finding of "no evidence of carcinogenicity…appears to be reasonable" (USFS 2011); however, this assessment was completed before EPA and IARC issued their cancer classifications described elsewhere in this table.
International Agency for Research on Cancer (IARC)	**Background:** IARC is the agency within WHO that, among other functions, issues monographs that classify toxic substances by human carcinogenic potential. IARC's glyphosate evaluation is a hazard assessment that considers whether glyphosate may cause cancer; it is not a risk assessment that quantifies the likelihood of developing cancer under specific circumstances. In 2015, IARC completed its hazard assessment evaluating carcinogenicity for glyphosate; its monograph was published in 2017 (IARC 2017). In January 2018, IARC issued a response to criticisms of its evaluation (IARC 2018).
	Determination: IARC's monograph concludes that glyphosate is "probably carcinogenic to humans (Group 2A)." The overall evaluation is based on "limited evidence in humans" for a positive association with non-Hodgkin lymphoma and "sufficient evidence in animals" (IARC 2017). IARC's evaluation of epidemiological data was based on many studies, including seven reports from the AHS cohort, a pooled analysis of three case-control studies in the Midwest U.S.; and multiple other case-control studies. IARC also determined that there was "strong evidence that exposure to glyphosate or glyphosate-based formulations is genotoxic based on studies in humans in vitro and in vivo animal studies" (IARC 2017).
Agricultural Health Study (AHS)	**Description**: AHS is an ongoing prospective cohort study that examines adverse health effects among pesticide applicators and their spouses. The National Cancer Institute (NCI) and NIEHS fund this study, which has included collaboration from EPA and NIOSH. With more than 89,000 participants since 1993, AHS is an extremely large epidemiological study. Although the study is not specific to glyphosate, the investigators have published journal articles on relationships between non-cancer and cancer outcomes and glyphosate use (e.g., Chang et al. 2023b; Islam et al. 2022; Parks et al. 2022; Andreotti et al., 2018; Meyer et al. 2017; Dennis et al. 2010; Andreotti et al. 2009; Lee et al. 2007; De Roos et al., 2005; Engel et al. 2005; Flower et al. 2004).
	Determination: Results from AHS studies varied based on specific details of the analyses conducted, such as how exposure is defined and characterized, the health outcome of interest, the statistical models used, and how the study population is defined. Results of earlier AHS studies were considered in the assessments conducted by other agencies. Sections 2.5.4 to 2.5.10 evaluates the more recent AHS studies.
National Toxicology Program (NTP)	**Background:** NTP issues cancer classifications for selected hazardous substances through its *Report on Carcinogens*. Although NTP has not yet classified glyphosate for carcinogenicity, the program is currently researching the toxicity of glyphosate and selected GBFs. In 1992, NTP conducted a series of short-term *in vivo* toxicity studies of glyphosate in the feed of mice and rats (Chan and Mahler 1992). More recently, NTP compared the cytotoxic and genotoxic effects of both glyphosate and GBFs using a suite of genetic toxicity and *in vitro*

Agency/Study	Background and Determination
	tests. Results from the genetic toxicity tests were recently published (Smith-Roe et al. 2023) and *in vitro* tests are ongoing.
	Determination: Results from the most recent rodent tests and *in vitro* genotoxicity tests indicated that glyphosate and its metabolite, AMPA, showed "no genotoxicity or notable cytotoxicity" in the concentration ranges tested (Smith-Roe et al. 2023). On the other hand, this research demonstrated that "all GBFs and herbicides [tested] other than glyphosate were cytotoxic, and some showed genotoxic activity" (Smith-Roe et al. 2023).
California Office of Environmental Health Hazard Assessment (OEHHA)	**Background**: California's OEHHA sets No Significant Risk Levels (NSRLs) for toxic substances regulated under the state's Safe Drinking Water and Toxic Enforcement Act of 1986 (i.e., Proposition 65). In July 2017, OEHHA issued an Initial Statement of Reasons document that proposed an NSRL for glyphosate (OEHHA 2021). A Final Statement of Reasons was issued in July 2022, which included a response to public comments (OEHHA 2022).
	Determination: OEHHA incorporated IARC's cancer classification for glyphosate. OEHHA set an NSRL of 1,100 micrograms per day (µg/day) based on the most sensitive animal study that OEHHA determined to be of sufficient quality, which was a study that found increased incidence of hemangiosarcomas observed in male CD-1 mice following two years of oral exposure to glyphosate technical.
European Food Safety Authority (EFSA)/European Chemicals Agency (ECHA)	**Background:** In 2017, following assessments by both EFSA and ECHA, glyphosate was reapproved for use in the European Union until December 15, 2022. This deadline has since been extended for one year. Four member states, which formed the Assessment Group on Glyphosate (AGG), recently submitted their glyphosate assessment report to both EFSA and ECHA (AGG 2021; 2022), who are now conducting a joint review of the AGG assessment. The AGG assessment reviewed literature through June 2020. The assessment process has undergone public comment and is undergoing peer review. The latest release of information is a high-level report from peer-review expert meetings in late 2022 (EFSA 2022). A final assessment is expected later in 2023.
	Determination: EFSA's 2015 assessment concluded that glyphosate is "unlikely to pose a carcinogenic hazard to humans" (EFSA 2015). This assessment also set an acceptable daily intake (ADI) and acute reference dose (aRfD) of 0.5 mg/kg/day, which was derived from a developmental toxicity study. In 2017, EFSA concluded that glyphosate does not have endocrine disrupting properties. In 2021, the AGG proposed classifying glyphosate as toxic but only for causing serious eye damage. In 2022, ECHA confirmed the original hazard classification for glyphosate (serious eye damage) and concluded that "classifying glyphosate as a carcinogenic, mutagenic or reprotoxic substance is not justified" (ECHA 2022).
Food and Agriculture Organization (FAO)/World Health Organization (WHO)	**Background:** In May 2016, FAO of the United Nations and the Core Assessment Group on Pesticide Residues of WHO convened a joint panel to evaluate human health risks of consuming food products that contain pesticide residues; a summary report was issued later in the year. This evaluation considered health risks for three pesticides, including glyphosate.
	Determination: The panel found that both short-term and long-term exposures to glyphosate residues in food are unlikely to present a risk to consumers (FAO/WHO 2016). FAO/WHO also concluded that glyphosate is unlikely to pose a carcinogenic risk to humans via exposure from the diet. An aRfD was not calculated, and an ADI was calculated based on effects on the salivary glands in rats, though the panel acknowledged "that these effects may be secondary to local irritation due to the low pH of glyphosate in solution."
Canada Pest Management Regulatory Agency (PMRA)	**Background:** PMRA of Health Canada authorizes uses of pesticides. In 2017, PMRA reauthorized use of glyphosate and published an assessment that considered cancer risk and potential health impacts associated with dietary exposures, occupational exposures, and household uses (PMRA 2017). An advocacy group sued the agency regarding the reauthorization decision; and in February 2022, a Federal Court of Appeal in Canada issued a ruling that directed the PMRA to reconsider certain procedural aspects of its evaluation; however, the court decision has not changed the glyphosate authorization.

Agency/Study	Background and Determination
	Determination: PMRA's 2017 assessment concluded that "the products containing glyphosate do not present unacceptable risks to human health…when used according to the revised product label directions" (PMRA 2017). The assessment summary further states: "The most sensitive endpoints for risk assessment were clinical signs of toxicity, developmental effects, and changes in body weight… However, the risk assessment approach ensures that the level of exposure to humans is well below the lowest dose at which these effects occurred in animal tests" (PMRA 2017).
Food Safety Commission of Japan (FSCJ)	**Background:** In 2016, Japan's FSCJ completed a human health risk assessment of different commercial grades of glyphosate. The complete assessment is only available in Japanese, but a summary of conclusions is written in English (FSCJ 2016). The human health risk assessment considered a range of cancer and non-cancer outcomes and derived an acceptable daily intake for glyphosate.
	Determination: The FSCJ assessment concluded (a) that "major adverse effects of glyphosate were observed on reduced gain of body weight, GI [gastrointestinal] tract, and liver"; and (b) that "glyphosate had no neurotoxicity, carcinogenicity, reproductive toxicity, teratogenicity, and genotoxicity" (FSCJ 2016). FSCJ also established an acceptable chronic daily intake (ADI) value of 1 mg/kg/day. FSCJ found it "unnecessary to specify an acute reference dose (aRfD)" because the lowest no-observed adverse effect level (NOAEL) dose in acute toxicity studies was higher than the Commission's reported "cut off level" (500 mg/kg/day).
Australian Pesticides and Veterinary Medicines Authority (APVMA)	**Background:** APVMA in Australia has multiple mandates, including regulation of the use of pesticides. In 2016, APVMA issued a regulatory position paper.
	Determination: APVMA's regulatory position paper found no "scientific grounds for placing glyphosate and products containing glyphosate under formal reconsideration," based on a reevaluation of human health data. APVMA also concluded that "exposure to glyphosate does not pose a carcinogenic or genotoxic risk to humans" (APVMA 2016).

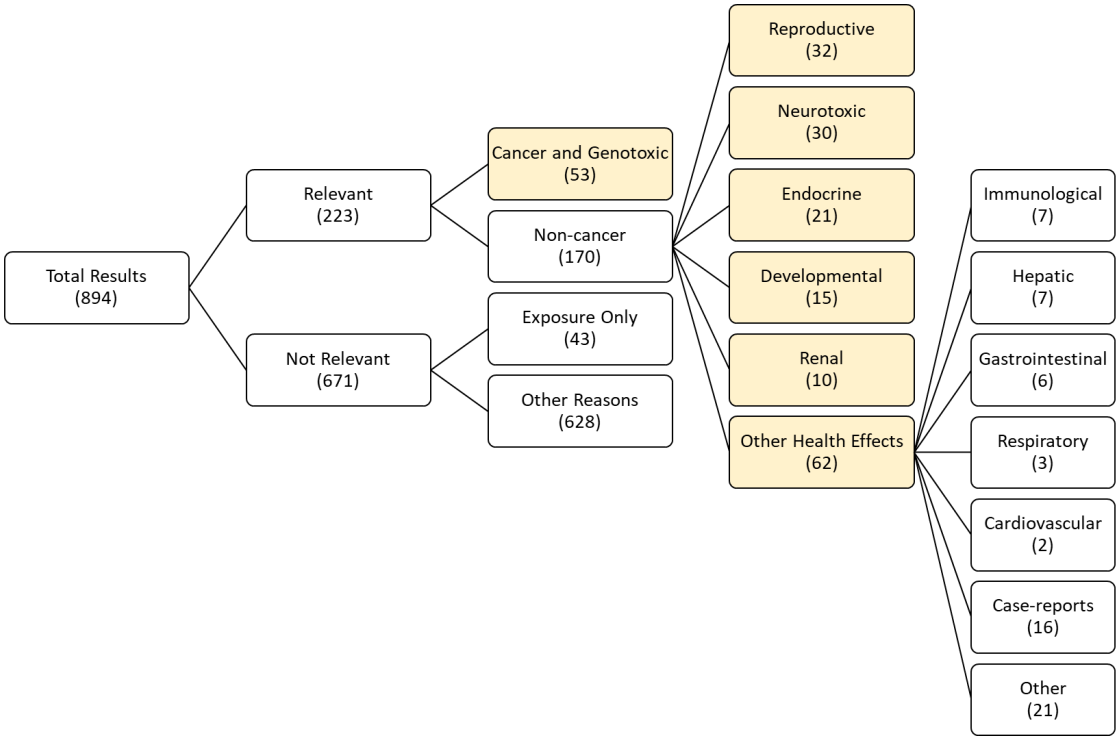
2.5.3 Peer-Reviewed Publications

ERG performed a supplemental literature search to identify recent peer-reviewed publications on glyphosate's human health impacts. The identified literature supplements our review of assessments published by recognized authorities, which were completed in different years and considered different cutoff dates for scientific literature. This scientific review's literature search was meant to identify articles of significance that were published following the assessments' most recent literature cutoff dates.

The search was conducted on January 12, 2023, and it focused on identifying articles published after January 1, 2019, on glyphosate-related cancer and non-cancer toxicity. ERG used a literature search string based on PubMed's Medical Subject Headings (MeSH) thesaurus and other PubMed tags. The search string (included in this report as Appendix A) used a combination of strings representing the chemical name, date, and health outcomes.

The literature search initially identified 894 articles. ERG downloaded titles, abstracts, and metadata for all articles into EndNote software. ERG then removed duplicate entries and articles not written in or translated into English. Next, ERG screened articles for relevance and potential impact by reviewing titles and abstracts. Following this initial review, 75 percent of articles were deemed not relevant for this project's evaluation of human health effects, primarily because the articles focused on non-mammalian species. Relevant articles were classified into categories based on the primary health outcome considered in each study. Some studies evaluated multiple health outcomes. Figure 2 summarizes the literature search results.

FIGURE 2. NUMBER OF UNIQUE ARTICLES RESULTING FROM LITERATURE SEARCH BY PRIMARY HEALTH OUTCOME



2.5.4 Cancer Effects

Information on glyphosate carcinogenicity is based on findings from major scientific assessments and on 28 recent (2019-2023) peer-reviewed publications related to cancer. In addition, this section summarizes 25 articles that describe findings related to glyphosate's genotoxicity, epigenetics, and other effects on DNA.

Review of findings from major scientific assessments. In 2015, IARC completed an assessment evaluating carcinogenicity for five pesticides and herbicides, including glyphosate. IARC's monograph, dated 2017, concludes that glyphosate is "probably carcinogenic to humans" (Group 2A) (IARC 2017). IARC reported "limited evidence in humans for the carcinogenicity of glyphosate," but also noting "statistically significant increased risks for NHL [non-Hodgkin lymphoma] in association with exposure to glyphosate" in multiple studies (IARC 2017). Glyphosate exposure was not directly measured in the underlying studies, and different surrogates for exposure (e.g., use of GBFs) were used instead. IARC also reported "sufficient evidence in experimental animals for the carcinogenicity of glyphosate." In 2018, IARC issued a written response to criticisms of its glyphosate findings (IARC 2018).

IARC's evaluation of epidemiological data was based on many studies. It identified certain studies as "key investigations" in part because of their relatively large size. Some of the key investigations included the AHS (De Roos et al. 2005), a pooled analysis of three case-control studies in the Midwest United States (De Roos et al. 2003), and a cross-Canada study (McDuffie et al. 2001). Many other case-control studies were also described. Across these studies, IARC identified four case-control epidemiological studies that reported statistically significant increased NHL risks associated with metrics used to characterize exposure to glyphosate, though the studies did not measure glyphosate exposure directly. IARC determined that the weight of evidence for multiple myeloma was not as strong, because the positive associations reported in the three studies were not statistically significant.

IARC's review of animal data was based on two studies in mice and six studies in rats, all of which considered oral exposure to glyphosate, whether in the diet or drinking water. The findings across the studies varied in terms of types of cancers that were increased and in which sexes; and in some studies, statistically significant increases in cancer were not observed. IARC also acknowledged various other data that supported its finding, such as "strong evidence that exposure to glyphosate or glyphosate-based formulations is genotoxic" (IARC 2017).

In contrast to IARC's cancer classification, every other major agency assessment reviewed for this report did not classify glyphosate as a carcinogen, as described below; some agencies continue to evaluate this issue. The following text summarizes the other agency assessments, and the review of more recent literature (later in this section) comments on why the various agencies reached different conclusions regarding glyphosate carcinogenicity.

EPA's 2017 draft human health risk assessment concluded that glyphosate technical and its metabolites are "not likely to be carcinogenic to humans" (EPA 2017b). EPA's determination was based on a comprehensive review of "epidemiological, animal carcinogenicity, genotoxicity, metabolism, and mechanistic studies" (EPA 2017b) that were incorporated into a weight of evidence analysis. Details on the evaluation are described in the *Revised Glyphosate Issue Paper: Evaluation of Carcinogenic Potential* (EPA 2017c). Since releasing this issue paper, EPA has reviewed and commented on more recent publications submitted through the public comment process (EPA 2017d; 2018; 2020b). These subsequent actions have not changed EPA's cancer determination. Key documents related to EPA's cancer assessment are described below:

- EPA's 2017 cancer evaluation, which includes findings from the draft human health risk assessment and the *Revised Glyphosate Issue Paper*, considered studies that were included in IARC's, FAO/WHO's, and ECHA/EFSA's assessments. This evaluation was based on both epidemiological data and animal data, as summarized below.
    - EPA's review of the epidemiological data included three studies deemed to be "high" quality, nineteen studies of "moderate" quality, and 34 studies of "low" quality. The three high-quality studies were two prospective cohort studies from the AHS (De Roos et al. 2005; Koutros et al. 2013) and a population-based case-control study in Sweden (Eriksson et al. 2008). EPA's assessment also included a recently updated AHS study (Andreotti et al. 2018). EPA's carcinogenicity evaluation states that "there was no evidence of an association between glyphosate exposure and solid tumors, leukemia, or HL [Hodgkin Lymphoma]" and notes that this is consistent with IARC, EFSA, and FAO/WHO assessments (EPA 2017c). EPA provided further context on its NHL evaluation. While acknowledging some evidence of associations between indicators of glyphosate exposure and NHL, EPA could not rule out the possibility that the reported associations could have been explained by chance or bias. Given conflicting results in the literature and limitations among the key studies, EPA ultimately found that "a conclusion regarding the association between glyphosate exposure and risk of NHL cannot be determined based on the available data" (EPA 2017c). EPA also describes limited evidence for multiple myeloma (MM) and concludes that the "available evidence does not link glyphosate exposure" to this disease (EPA 2017c).
    - EPA's review of animal data identified eight chronic rat studies and six mouse studies that met the Agency's acceptability criteria for evaluating glyphosate technical (EPA 2017b; 2017c). EPA noted several limitations associated with the information across the studies. For example, consistent findings across studies were not observed, and the findings from selected studies did not show monotonic dose-response, evidence of tumor progression, or evidence of pre-neoplastic lesions. Based on these and other considerations, EPA could not be certain that glyphosate technical caused the tumors observed in the studies.
- In EPA's response to comments on the draft human health risk assessment, EPA reported that none of the external peer reviewers on the science advisory panel "believe glyphosate should be classified as 'likely to be carcinogenic to humans' or 'carcinogenic to humans'" (EPA 2018a). EPA also characterized its assessment as "more robust" and "more transparent" than IARC's assessment. As an example, EPA notes that IARC considered only a subset of the animal carcinogenicity studies considered by EPA.
- During its comment-response process, EPA also evaluated two publications on glyphosate and NHL issued since the Agency completed its 2017 issue paper evaluating the carcinogenic potential of glyphosate (EPA 2020b). One publication was a meta-analysis published by Zhang et al. (2019a) that reported a statistically significant increase for NHL among GBF-exposed individuals and the other was a pooled analysis published by Leon et al. (2019) that did not find a statistically significant association for NHL but did find one for one subtype of NHL. Both studies are described in more detail in the recent literature section

below. Among other critiques, EPA questioned the meta-analysis methodology used by Zhang et al. (2019a). When EPA conducted its own meta-analysis using more recent cancer data from AHS, the Agency found that the association between indicators of glyphosate exposure and NHL incidence was not statistically significant. Leon et al. (2019) reported a statistically significant increase for diffuse large B-cell lymphoma, but EPA noted that the confidence interval for the reported odds ratio included 1.0. For both studies, EPA stated that the new results did not impact EPA's previous conclusions.

- In 2020, EPA finalized its latest registration decision for glyphosate and reconfirmed its human health findings (EPA 2020a). While the decision has been withdrawn following a legal challenge, EPA has recently stated that the withdrawal "does not automatically mean that EPA's underlying scientific findings regarding glyphosate, including its finding that glyphosate is not likely to be carcinogenic to humans, are either incorrect or cannot be used as support for a future decision" (EPA 2022b).

ATSDR's *Toxicological Profile for Glyphosate* summarized evidence of carcinogenicity in humans and in laboratory animals (ATSDR 2020). However, ATSDR generally does not issue its own cancer classifications and instead typically reports those issued by EPA, IARC, and NTP.

For humans, ATSDR reviewed 22 epidemiological studies that examined relationships between GBF use and cancer. Most of ATSDR's discussion focused on the evidence for lymphohematopoietic cancers, such as NHL. Overall, ATSDR summarizes that "numerous studies reported risk ratios greater than one for associations between glyphosate exposure and risk of non-Hodgkin lymphoma or multiple myeloma; however, the reported associations were statistically significant only in a few studies" (ATSDR 2020). For the lymphohematopoietic cancers, ATSDR reviewed six meta-analyses and numerous case-control and other epidemiological studies. The meta-analyses included the review documented in IARC's monograph; two others that were completed before IARC published its monograph (Schinasi and Leon 2014; Chang and Delzell 2016); and three analyses completed after IARC published its monograph. The three more recent analyses are described below in the recent literature section (Leon et al. 2019; Pahwa et al. 2019; Zhang et al. 2019a). Five of the six meta-analyses reported significant associations between indicators of glyphosate exposure and NHL. Some also reported associations with MM (Chang and Delzell 2016) and the NHL subtype, diffuse large B-cell lymphoma (Pahwa et al. 2019). The studies that ATSDR reviewed did not find significant associations between glyphosate exposure and various solid-type tumors.

For animals, ATSDR reviewed seven publications on *in vivo* studies on the carcinogenic potential of glyphosate technical or GBF. EPA authored five of these publications, which summarized results from *in vivo* tests, including some that are not publicly available (e.g., tests submitted by the glyphosate registrant). ATSDR also describes findings from two *in vivo* studies conducted to assess the potential for glyphosate or GBF to cause MM. One was an initiator-promoter study involving the dermal administration of GBF to albino mice; it reported that the GBF was a tumor promoter but not an initiator (George et al. 2010). The other investigated the potential for glyphosate to cause MM in rats (Wang et al. 2019a) and is described in more detail below.

In 2015, EFSA/ECHA concluded that glyphosate is "unlikely to pose a carcinogenic hazard to humans and the evidence does not support classification with regard to its carcinogenic potential" (EFSA 2015). This finding was based on a peer review organized by EFSA, in which reviewers made conclusions regarding the evidence for carcinogenicity in humans and in laboratory animals. For humans, most of the peer reviewers reported finding "very limited" evidence for associations between GBF exposure and NHL. However, some minority views noted "limited" evidence or "inadequate" evidence of an association. For animals, all but one of the peer reviewers reported finding no evidence of carcinogenicity among the rat and mice studies considered. Various reasons were provided for this finding, such as the lack of consistent results across studies and the observed effects falling within the historical range for controls.

EFSA/ECHA have continued to review the human health concerns of glyphosate and have responded to public concerns about IARC's assessment results. While EFSA/ECHA are expected to issue a final risk assessment later in 2023, ECHA has reconfirmed its original hazard classification for glyphosate. On May 30, 2022, ECHA released a statement concluding that, based on the scientific evidence, "classifying glyphosate as a carcinogenic, mutagenic or reprotoxic substance is not justified" (ECHA 2022). Additionally, a high-level report of minutes from a recent peer-review expert meeting released on December 25, 2022, indicates that EFSA will maintain ECHA's cancer determination by concluding that "the available epidemiological studies currently do not provide sufficient

indication that glyphosate exposure is associated with any cancer-related health effect" (EFSA 2022). Minutes from the December 2022 peer review note that the experts will still consider results from a recent study, De Roos et al. (2022). This study is described in more detail below.

The FAO/WHO joint meeting concluded that "glyphosate is unlikely to pose a carcinogenic risk to humans via exposure from the diet" (FAO/WHO 2016). This conclusion was based on evidence in humans, animals, and genotoxicity studies. Following a review of the epidemiological data, FAO/WHO concluded: "Overall, there is some evidence of a positive association between glyphosate exposure and risk of NHL from the case-control studies and the overall meta-analysis; however, it is notable that the AHS, which is the only cohort study and is large and of high quality, found no evidence of association at any exposure level." In animals, FAO/WHO reviewed nine carcinogenicity studies in mice and eleven in rats. The assessment cited "equivocal evidence" of lymphomas in male mice (three studies) and female mice (one study), and these studies considered doses of at least 814 mg/kg/day. The assessment noted, however, that three other studies that considered higher doses found no evidence of lymphoma. In rats, the assessment concluded "no reliable evidence for treatment-related tumours." The meeting also concluded that glyphosate is "unlikely to be genotoxic at anticipated dietary exposures."

The assessments issued by USFS (USFS 2011), Health Canada (PMRA 2017), FSCJ (FSCJ 2016), and APVMA (APVMA 2016) all similarly concluded that glyphosate is not carcinogenic and unlikely to pose a human cancer risk.

Review of recent (2019-2023) peer-reviewed studies examining cancer in humans. ERG's literature search identified eighteen recent publications that evaluated human populations exposed to GBFs to assess relationships between glyphosate and cancer. This includes recent epidemiological studies, multiple meta- or pooled analyses, critiques and responses to certain publications, and editorial commentaries.

Review of Human Data for NHL and MM

Many recent review articles, including multiple meta-analyses and pooled analyses, have investigated the associations between glyphosate and NHL. Conflicting results have prompted research groups to respond to each other's work via the peer-reviewed publication process. Because these articles build upon (and often directly respond to) one another, the following review articles are described in chronological order of publication. Note that in some instances, authors reported associations between "glyphosate" and NHL/MM, even though the study population was presumably exposed to GBFs, not glyphosate technical.

- Zhang et al. (2019a) investigated the association between GBFs and NHL. Based on a literature search considering publications up through August 2018, the authors identified six studies for evaluation: one was updated results to the AHS study and five were case-control studies. Taken together, these studies evaluate cancer outcomes in nearly 65,000 individuals from the United States, Canada, Sweden, and France. Three out of the six studies found significant odds ratios above 1 for NHL. This meta-analysis chose from each study the relative risk (RR) or odds ratio (OR) corresponding to the highest cumulatively exposed group. A logistic regression analysis resulted in a meta-RR of 1.41 (95% CI: 1.13-1.75), which indicates a significant 41 percent increased risk of NHL for high cumulative exposure to GBFs. The authors conducted various sensitivity analyses, including varying measures of exposure and applying different study inclusion-exclusion criteria. The meta-RR remained significantly above 1 in each sensitivity analysis.
- Leon et al. (2019) conducted a pooled analysis of three cohorts of agricultural workers to investigate associations between pesticide use and NHL. The analysis included 315,250 farmers and 2,430 NHL cases from France, Norway, and the United States. Ever-use exposure of fourteen pesticide chemical groups and 33 active ingredients was collected in two ways: for the European cohorts, it was derived from self-reported history of crops cultivated and a crop-exposure matrix; for the United States cohorts, it was derived from self-reported lifetime use. The statistical analysis did not find a significant relationship between use of pesticides containing glyphosate (GBFs) and NHL (hazard ratio = 0.95; 95% CI: 0.77-1.18) but did for diffuse large B-cell lymphoma (hazard ratio = 1.36; 95% CI: 1.00-1.85).
- Pahwa et al. (2019) is a pooled analysis of data from the North American Pooled Project (NAPP) that investigated the association between glyphosate exposure and NHL and its subtypes. The NAPP study includes case-control studies conducted in four Midwestern states and in six Canadian provinces. Self-reported exposure to glyphosate in pesticides was characterized by duration, frequency, cumulative use,

and "ever-use." Unconditional multiple logistic regression was used to calculate ORs for associations between the different exposure metrics and NHL and its subtypes. Analyses also considered confounding by other pesticides. For NHL, the meta-analysis OR was 1.43 for the ever-use of glyphosate in pesticides, and this result was statistically significant (95% CI: 1.11–1.83). Significant relationships were also observed between the ever-use of glyphosate in pesticides and other cancer subtypes—diffuse large B-cell lymphoma (DLBCL) and "other"; however, after controlling for the "ever-use" of three other pesticides (2,4-D, dicamba, and malathion), the ORs for glyphosate were no longer significant. The authors investigated other exposure metrics. When comparing participants with two or fewer days of using GBFs per year to participants with more than two days of use, the association between NHL and DLBCL was significant, even after controlling for confounders.

- Crump (2020) is a perspective article that discusses the potential for recall bias and selection bias in case-control studies investigating relationships between glyphosate and cancer. Crump notes how these potential biases can affect epidemiological studies in general, and he explains how the biases could have impacted glyphosate studies. The recall bias results from exposure characterizations based on participants' recollections of their own pesticide use. Crump suggests that cases, when compared to controls, might be more likely to recollect past glyphosate use. He also describes a form of selection bias that may exacerbate the recall bias in two of the case-control studies; in some odds ratios, these studies excluded controls who were not exposed to glyphosate but who were exposed to other pesticides, but a similar exclusion did not apply to cases.
- Donato et al. (2020) conducted a meta-analysis that investigated the effects of occupational exposure to glyphosate and risk of NHL and MM. The research included relevant occupational epidemiological studies published through May 15, 2019; six articles were identified for NHL, and three for MM. Random effects model results showed that ever-exposure to glyphosate resulted in a meta-RR of 1.03 (95% CI: 0.86-1.21) for NHL and 1.04 (95% CI: 0.67-1.41) for MM. The authors conducted various sensitivity analyses including removing each individual study and only including the highest category of exposure in the subset of studies with dose-response data. These results did not change the significance of the risk estimates.
- Authored by the research team that published the Zhang et al. (2019a) study, Rana et al. (2020) sought to explain why the meta-analyses documented in Donato et al. (2020) and Zhang et al. (2019a) reached different conclusions. Overall, the analyses described in Rana et al. (2020) do not support the findings previously reported by Donato et al. (and nearly all of Donato et al.'s calculations reportedly could not be replicated). Further, Rana et al. (2020) found that the increased NHL cancer risk previously reported in Zhang et al. (2019a) might be an underestimate of the actual risk. One issue cited in the discussions was the importance of the selected exposure metric, which Kabat et al. (2021) discusses further (see next item).
- Kabat et al. (2021) conducted a sensitivity analysis of Zhang et al.'s (2019a) meta-analysis using alternate measures of exposure. They note that the risk estimates in Zhang et al. were, among other things, inconsistent across studies and that the highest available estimates were selected in all studies. Kabat et al. reanalyzed the same studies but varied the choice in exposure estimates from each study. They also calculated new meta-risks for "ever-exposure" to glyphosate. The results of their analysis confirm that Zhang et al.'s choice of a 20-year lag and the highest exposure level from each study resulted in the highest meta-RR of 1.41. If a fifteen-year lag was chosen instead, then the RR would be 1.25 and still statistically significant (95% CI: 1.01–1.25). When the exposure metric chosen is "ever-exposure," the results are no longer significant. The authors conclude that "the results of meta-analyses of glyphosate exposure and NHL risk depend on assumptions made about both exposure level and latency period."
- Boffetta et al. (2021) is a response to Rana et al. (2020) by the authors of Donato et al. (2020). In this paper, the authors updated their original analysis by using log-transformed effect measures and expanding the literature search date through December 2020, which added a recent study to the meta-analysis. The updated meta-analysis found that ever-exposure to glyphosate resulted in a meta-RR of 1.05 (95% CI: 0.90-1.24). Sensitivity analyses were conducted that did not change the results. The authors also updated their analysis of lymphoma subtypes and found that the meta-RR for DLBCL was significant at 1.21 (95% CI 1.02-1.63). An analysis restricted to the highest category of exposure was not significant, but it only included three studies.

- Weisenburger (2021) reviews findings on the link between GBFs and NHL considering epidemiological studies, meta-analyses, and pooled analyses published since IARC completed its assessment. In addition to considering the epidemiological data and associated re-analyses, this review describes animal studies, mechanisms of carcinogenicity, and evidence for causation. The authors conclude that "the epidemiologic, animal, and mechanistic studies together provide a coherent and compelling pattern of evidence that glyphosate and GBFs are a cause [of] NHL in humans exposed to these agents."
- Tarone (2022) is a letter to the editor in reply to Weisenburger (2021). The letter takes exception to how Weisenburger characterized some of Tarone's earlier work as "industry-sponsored" and concludes that "there is simply no credible evidence that glyphosate causes malignancies in rodents."
- De Roos et al. (2022) is a pooled analysis of data from ten case-control studies that investigated the association between occupational pesticide use and NHL. The ten studies include populations in Australia, Canada, Europe, and the United States. Occupational use of GBFs were derived from participants' questionnaire responses or assigned by experts in the individual studies. Exposure metrics included ever-use, duration, and lagged use. Logistic regressions were used to calculate ORs and to control for confounders, including other pesticide exposures. Results showed that the meta-OR of 1.03 for ever-use of pesticides containing glyphosate was not significantly associated with NHL (95% CI: 0.83-1.29) in the analyses that controlled for other pesticide exposures. Authors note that a consistent result across analyses was an association between glyphosate exposure lagged 10 years and follicular lymphoma; however, this result was not statistically significant (OR=1.48, 95% CI: 0.98-2.25).

Two recent epidemiological studies (non-reviews) investigated the association between glyphosate exposure and lymphomas. The authors of these studies reported associations between "glyphosate" and certain cancers, even though the study population was presumably exposed to GBFs, not glyphosate technical.

- A case-control study conducted in Italy evaluated the association between the risk of lymphoma subtypes and "semi-quantitative indicators" of occupational exposure to glyphosate (Meloni et al. 2021). The study included 867 incident lymphoma cases between 2011 and 2017 and 774 matched controls from multiple medical centers. The frequency, duration, intensity, and confidence of occupational exposure to glyphosate was estimated based on expert evaluation of a comprehensive questionnaire. Results showed that only 2.2 percent of participants were classified as ever exposed to glyphosate, and this group had a 4.5- to 12-fold elevated risk of follicular lymphoma depending on the exposure metric (e.g., "ever exposed to glyphosate with medium-high confidence," "medium-high cumulative exposure level," etc.). These results are based on four cases of follicular lymphoma. No association was observed for other lymphoma subtypes.
- A cross-sectional study evaluated estimates of exposure to agricultural pesticides for 35,808 NHL patients in the California Cancer Registry (Poh et al. 2022). Exposure estimates for five individual active ingredients (including glyphosate) at the census tract level were derived from California's Pesticide Use Reporting data, land-use data, and the CalEnviroScreen tool. Cumulative exposure estimates were calculated from ten years before diagnosis to one year after. No significant association was observed between any pesticide and lymphoma-specific and overall survival.

Review of Human Data for Other Cancers

Three recent epidemiological studies investigated the association between glyphosate exposure and other cancers. Once again, the study populations were presumably exposed to GBFs (and not glyphosate technical), even if the articles did not clearly make this distinction.

- A case-control study examined the association between exposure to 29 pesticides and thyroid cancer (Omidakhsh et al. 2022). The study included 2,067 cases of thyroid cancers from the central California agricultural area and 1,003 controls from the same area. As in Poh et al. (2022), California's Pesticide Use Reporting data and land-use data were used to estimate residential exposure to agricultural pesticides in pounds per year within 500 meters of each residence. Estimated exposure to ten different pesticides, including glyphosate compounds, was significantly associated with increased odds of thyroid cancer in single pesticide models (OR: 1.33, 95% CI: 1.12-1.58). Pesticide exposures were highly correlated with one

another, and the only pesticide that was consistently associated with thyroid cancer in multiple pollutant models was paraquat dichloride. In models that excluded paraquat dichloride exposure, glyphosate exposure was no longer associated with thyroid cancer.

- A pilot nested case-control study explored the association between pre-diagnostic urinary AMPA excretion and breast cancer risk in 250 predominantly postmenopausal women in Hawaii (Franke et al. 2021). The study included 124 cases and 126 healthy controls. AMPA was detected in 87 percent of participants. Results showed that after adjusting for covariates, urinary AMPA excretion was nearly 38 percent higher among cases than controls. This difference corresponded to an increased risk of developing breast cancer (4.5-fold) in the highest quintile of AMPA excretion compared to the lowest. The authors concluded that their research was "the first study to prospectively examine associations between urinary AMPA excretion and breast cancer risk."
- The literature search also identified three studies (Feulefack et al. 2021; Khan et al. 2022a; Khan et al. 2022b) that investigated the relationship between parental exposures to pesticides and certain childhood cancers. The studies are not discussed here because they did not characterize glyphosate exposures.

Review of recent literature (2019-2023) on cancer in laboratory animals. ERG identified four recent publications related to cancer in laboratory animals: an *in vivo* study, a pooled analysis of animal studies, a review article, and a perspective article.

The *in vivo* study dosed mice with glyphosate technical and investigated blood and bone indicators potentially linked to NHL and MM (Wang et al. 2019a). Wildtype mice and mice from a genetic line described as "the best available animal model for MM" were dosed with glyphosate technical via drinking water at 1,000 mg/L for 72 weeks, at which point blood and organs were collected. Among other findings, results showed that glyphosate-exposed mice from the MM genetic line developed progressive hematological abnormalities and plasma cell neoplasms and had high serum IgG. Authors concluded that the data supported a B-cell-specific mechanism for glyphosate-induced NHL and MM carcinogenesis.

A pooled analysis compiled literature on *in vivo* animal studies to characterize the potential carcinogenicity of glyphosate technical (Portier 2020). The review evaluated carcinogenicity animal data from 21 chronic rodent studies (thirteen on rats and eight on mice) that were identified in published literature, agency assessments (EPA, EFSA, and FAO/WHO), and industry studies released through judicial proceedings. The author found thirteen of the 21 studies to be of sufficient quality for inclusion in a pooled analysis. Results identified 37 significant tumor findings and that the data "demonstrate consistency across studies in the same sex/species/strain for many of these tumors."

Berry (2020) is a perspective article that discusses the evidence for glyphosate's carcinogenicity in the context of conflicting agency assessments. The author reviewed the evidence for bioassays, genotoxicity studies, and animal studies and found that the evidence is insufficient to determine that glyphosate is carcinogenic. The author notes that IARC's determination was based on multiple observations of trends that were not dose-dependent, not present in pair-wise comparisons, and were limited to a single sex in individual rodent studies. Berry also notes that some cancer types observed (e.g., hepatocellular neoplasms in mice, certain thyroid tumors in rats) are common in long-term studies of rodents.

A review article discussed potential statistical issues in IARC's review of *in vivo* studies of rodents (Crump et al. 2020). The authors argue that IARC did not account for the large number of statistical tests performed and the likelihood of some significant associations occurring by chance. The authors reanalyzed the *in vivo* study data using a methodology that considers false-positive probabilities. The authors conclude that "these tests found no strong or convincing evidence that glyphosate is an animal carcinogen."

Review of recent literature (2019-2023) of in vitro studies using cancer cell lines. Four recent studies involved investigating the effects of glyphosate technical, GBFs, or both on cancer cell lines. One found that epithelial-mesenchymal transition was induced in a human endometrial carcinoma cell line when exposed to both glyphosate technical and 17β-estradiol (E2) (Gastiazoro et al. 2020). Another found that glyphosate technical exposure did not alter the global proteome of a breast cancer cell line (Antoniou et al. 2019). A third study of a benign breast cell line found that glyphosate technical exposure was associated with a significant reduction in DNA

methylation, though tumors did not develop (Duforestel et al. 2019). The fourth study found that GBF-induced gene expression changes in the cell cycle and DNA damage repair pathways in a breast cancer cell line (Stur et al. 2019).

Review of recent literature (2019-2023) on genotoxic, epigenetic, and other effects on DNA. 27 recent studies involved investigating genotoxic, epigenetic, oxidative stress, and other effects on DNA of glyphosate technical, GBFs, and AMPA. These studies are briefly described because of the link between genotoxicity and carcinogenicity; however, not all genotoxic effects lead to cancer, and not all cancers are due to genotoxic effects.

Two recent epigenome-wide association studies were conducted in humans:

- A case-control study nested within the AHS investigated the association between pesticide exposures and epigenetic effects in farmers (Hoang et al. 2021). Blood was collected from 1,170 farmers and analyzed for DNA methylation. Results from participants with past and recent use of nine pesticide active ingredients were compared to results from participants who never used these pesticides. Specific epigenetic markers (differential C-phosphate-G sites) were identified for the use of GBFs.
- A cross-sectional study investigated the association between DNA methylation in white blood cells and glyphosate and AMPA biomarkers in women (Lucia et al. 2022). Blood and urine were collected from 392 postmenopausal women. Glyphosate and AMPA were measured in urine, and DNA methylation was evaluated in white blood cells. Among other findings, urinary glyphosate was associated with methylation in 24 CpG sites and AMPA was associated with methylation in four sites. A methylation index was calculated and used to predict urinary glyphosate levels in a validation set.

Two studies investigated oxidative stress biomarkers in humans, which have been associated with some cancers:

- In a sub-cohort of the AHS, Chang et al. (2023b) examined the relationship between urinary glyphosate concentrations, recent use of glyphosate-based pesticides, and urinary oxidative stress biomarkers. First-morning void urine samples were collected from 268 male farmers who self-reported recent and lifetime occupational use of pesticides containing glyphosate and from 100 matched male non-farmers. Results showed that farmers with urinary glyphosate levels in the highest quartiles had elevated oxidative stress biomarkers (8-OHdG and malondialdehyde [MDA]) compared to those in the lowest quartile, both in pair-wise comparisons and in trend tests across quartile means. Among farmers who used pesticides containing glyphosate the day before urine samples were collected, these biomarkers were also statistically elevated.
- A cross-sectional study of children in Cyprus investigated the association between urinary glyphosate and biomarkers of oxidative stress (Makris et al. 2022). Urine samples from 177 children aged 10 to 11 years were analyzed for glyphosate, AMPA, metabolites of other pesticides, and oxidative stress biomarkers of lipid and DNA damage. Pesticide use information was reported by parents. Median urinary glyphosate levels were below limits of quantification, and median creatinine-adjusted AMPA levels were 0.18 ng/mL. A statistically significant association was observed between urinary AMPA and the biomarker for DNA damage (8-OHdG), but no statistically significant association was observed for urinary glyphosate. Similar associations were observed with the other pesticides measured in this study, but co-pesticide exposure sensitivity analyses were not conducted.

Four *in vivo* studies looked at epigenetic effects via DNA methylation following exposures to glyphosate technical, GBFs, or both:

- No significant changes in global DNA methylation and expression of specific DNA methylation enzymes were observed in the small intestine, kidney, or liver of pigs fed glyphosate technical at 20 ppm and 200 ppm (Larsen et al. 2022); however, a significant increase in expression of an enzyme responsible for demethylation was observed in the kidneys of pigs in the higher treatment group.
- Increased expressions of DNA methyltransferase genes were observed in the liver and kidney of rats dosed with Roundup (Ergun et al. 2021).

- Gestating female rats were exposed to glyphosate technical and epigenetic biomarkers (DNA methylation regions and histone retention sites) associated with observed diseases were identified in the sperm of great-grand (F3) male offspring (Maamar et al. 2021; Beck et al. 2022).

Two review articles reviewed the *in vivo* and *in vitro* evidence linking glyphosate technical to a range of epigenetic effects (Bukowska et al. 2022; Rossetti et al. 2021). Observed effects included global methylation across cell types and organisms, methylation of ERα, epigenetic modifications to specific histones, changes in the expression of different protein genes in humans, and changes in the regulation of microRNAs. The authors note that epigenetic modification can result in inherited disorders across generations, but they said further research is needed to link epigenetic modifications to specific diseases. A third review article summarized evidence that glyphosate causes oxidative stress and discussed the mechanism by which this occurs (Wang et al. 2022).

Four *in vitro* studies evaluated DNA methylation following exposure to glyphosate technical, AMPA, or both. These studies found a range of genotoxic effects of glyphosate exposure. Woźniak et al. (2021) observed that both glyphosate technical and AMPA altered the expression of genes involved in DNA methylation and in histone deacetylation. The same research group also observed the effects of glyphosate technical and AMPA on the reduction of global DNA methylation and methylation of tumor suppressor genes (Wozniak et al. 2020a; 2020b). Courant et al. (2022) observed a dose-dependent association between exposure to glyphosate technical (and several other chemicals) and expression of a protein-coding gene linked to DNA demethylation.

Twelve *in vitro* studies investigated the potential for glyphosate to directly cause DNA damage. The authors of multiple publications refer to "glyphosate" exposure, which is assumed to be glyphosate technical unless the publications clearly stated that a GBF was evaluated.

- Barron et al. (2022) observed increased markers of DNA damage at the highest glyphosate dose in human liver cells.
- Bhardwaj et al. (2019) and Bhardwaj et al. (2022b) both focused on the mitigating effects of vitamins C and E on cell damage following glyphosate exposure.
- Congur (2021) focused on exploring the combined effect of glyphosate and another herbicide, 2,4-D.
- Hao et al. (2019c) observed a dose-response association between GBF (Roundup) and increases in DNA damage in human alveolar carcinoma cells. The same research group tested differences between GBF, glyphosate technical, and an adjuvant (POEA) and observed various types of DNA damage (e.g., oxidative damage, single- and double-strand breakage) in the GBF- and POEA-treated cells that were not observed in the glyphosate technical treated cells (Hao et al. 2020b).
- Mesnage et al. (2021a) tested three herbicides on a panel of *in vitro* carcinogenic tests and found that, unlike the other two herbicides tested, glyphosate did not induce a positive outcome on any of the tests. The same research group tested a GBF (RangerPro), POEA, and glyphosate technical in the same panel of tests and found DNA damage, oxidative stress, and unfolded protein responses on the GBF and POEA assays, but not the assays run with glyphosate technical (Mesnage et al. 2022c).
- Nagy et al. (2019) tested three GBFs (Roundup Mega, Fozat 480 and Glyfos) and glyphosate technical for genotoxicity and found cytotoxicity and DNA damage in cells treated with the GBFs, but not in the cells treated with glyphosate technical. The same research group found that glyphosate technical increased micronucleus formation, though the GBFs exhibited greater toxicity (Nagy et al. 2021).
- Sonzogni et al. (2022) observed that glyphosate may induce DNA double-strand breaks in human skin fibroblast and brain cells.
- Tarboush et al. (2022) found that glyphosate only exhibited a significant increase in sister-chromatid exchange in cultured human lymphocytes at the highest dose tested; however, no association was observed for all other measured indicators of genotoxicity.

2.5.5 Reproductive Effects

Information on glyphosate reproductive toxicity is based on findings from major scientific assessments and on 32 recent (2019-2023) peer-reviewed publications identified in ERG's literature search. Some studies described in the developmental and endocrine sections also relate to reproductive toxicity.

Review of findings from major scientific assessments. In its 2017 draft human health risk assessment for glyphosate, EPA reviewed findings from three *in vivo* developmental toxicity studies conducted in rats (EPA 2017b). Two of them were two-generation studies, and the third was a three-generation study. EPA determined that the oldest study (from 1981) had spurious results (see Section 2.5.9 for further details). The lowest NOAEL observed across the other two studies was 1,234 mg/kg/day. EPA does not report a lowest observed adverse effect level (LOAEL), because the studies provided "no evidence of reproductive toxicity in the adults."

ATSDR's *Toxicological Profile for Glyphosate* summarized evidence of glyphosate reproductive toxicity in humans and in laboratory animals (ATSDR 2020). For humans, ATSDR considered findings from three epidemiological studies published between 1999 and 2017 that had mixed results for reproductive outcomes. A retrospective cohort study of women living on farms in Canada found no association between pesticide use and probability of conception (Curtis et al. 1999); an ecological study found no association between fertility and use of glyphosate pesticides in five regions of Colombia (Sanin et al. 2009). The third study considered associations between aerially sprayed glyphosate pesticides and miscarriage. While a positive association was reported, the study does not document the aerial application rates, nor does it consider ground-based applications of pesticides. Also, the study defines miscarriage in a manner that may have overestimated the effect (Camacho and Mejia 2017).

For animals, ATSDR reviewed five *in vivo* studies that examined reproductive effects following glyphosate technical exposure and thirteen *in vivo* studies that examined development effects following GBF exposures. The studies all considered oral exposure to different species of mice and rats. Across the studies, effects were more consistently observed in males than in females. ATSDR's review of the glyphosate technical studies found that "reproductive endpoints have been evaluated, but do not appear to be particular targets of glyphosate toxicity" (ATSDR 2020). For studies of GBFs, many effects were noted across studies, species, and sexes. ATSDR summarized the GBF studies by noting a LOAEL of 640 mg/kg/day for development of abnormal sperm in male mice, though some studies reported effects at lower doses. ATSDR also reviewed the results of *in vitro* studies that demonstrated the potential for reproductive toxicity in different cell lines.

EFSA's 2015 assessment considered many of the same underlying publications that factored into EPA's 2017 draft human health risk assessment. EFSA concluded in its report that glyphosate technical "is not classified or proposed to be classified as…toxic for reproduction category 2" (EFSA 2015). For reference, a category 2 classification is used for suspected human reproductive toxicants, generally based on evidence from humans or laboratory animals (EC 2008). EFSA concluded that glyphosate technical administration in animal studies did not affect reproductive or fertility parameters, except at high doses (LOAEL = 1,000 mg/kg/day; NOAEL = 351 mg/kg/day) (EFSA 2015). The report did not reach conclusions regarding reproductive toxicity of GBFs.

The FAO/WHO joint meeting reviewed potential effects from seven reproductive studies in rats, and some of these studies were also considered by EFSA (FAO/WHO 2016). While these studies reported toxicity for other health endpoints (e.g., gastrointestinal effects, developmental effects), FAO/WHO concluded that these studies provide "no evidence of reproductive toxicity" at doses up to 1,983 mg/kg/day, which was selected for the reproductive NOAEL (FAO/WHO 2016).

Three other assessments commented on reproductive toxicity of glyphosate. First, based on its review of several epidemiology studies that investigated health effects related to GBFs, USFS concluded that none has "demonstrated a statistically significant association between exposure and reproductive effects" in humans (USFS 2011). Second, FSCJ reviewed a two-generation reproductive study in rats that considered five different glyphosate technical grades and concluded that glyphosate "had no…reproductive toxicity" (FSCJ 2016). Third, the APVMA assessment found it "extremely unlikely" that glyphosate causes reproductive toxicity under normal conditions of exposure (APVMA 2016).

Review of recent (2019-2023) peer-reviewed studies examining reproductive toxicity in humans. ERG's literature search did not identify any recent epidemiological studies that evaluated glyphosate reproductive effects in humans. See Section 2.5.8 for a review of relevant developmental effects, including pre-term birth.

Review of recent literature (2019-2023) on reproductive toxicity in laboratory animals. ERG's literature search identified 19 recent publications that evaluated reproductive effects associated with exposures to glyphosate or

GBFs. The 19 publications were authored by researchers worldwide, including from the United States, Argentina, China, the Democratic Republic of Congo, Egypt, France, and Iran.

These publications included 15 laboratory studies that were conducted on rats (n=7), mice (5), sheep (1), pigs (1), and guinea pigs (1). This summary focuses on publications that had a primary focus of investigating treatment-related effects on animals; it does not summarize review articles that do not present original toxicology research (Milesi et al. 2021; Serra et al. 2021) or publications reporting on potential treatments to mitigate impacts of glyphosate exposure (Cao et al. 2021; Hashim et al. 2022).

The following paragraphs summarize the *in vivo* findings, organized by species and sex of the animals studied:

- *Findings in male rats.* Four publications reported on reproductive effects in male rats associated with exposures to glyphosate technical or GBFs:
    - One study exposed juvenile rats orally with glyphosate or GBF at doses of 0, 2, or 50 mg/kg/day from post-natal day fourteen to post-natal day 30, which is the period when testes develop fertility (Gorga et al. 2021). On the 31st day, blood and tissue samples were collected from most of the rats. A subset of rats in the highest exposed GBF group were retained through adulthood and sacrificed on day 90. No difference was observed between body and testis weight across groups; however, a significant difference was seen between treatment and control groups in the permeability of the blood-testis barrier. This difference was observed in all treatment groups, though not in a dose-dependent manner. No differences in various other parameters measured in the testis were observed (e.g., testosterone and various protein levels, androgen receptor expression). For the adult rats sacrificed on day 90, various measures of sperm quality, sperm quantity, and testis integrity were not different between exposed and control groups. The authors hypothesized that "blood-testis barrier impairment is a reversible phenomenon."
    - The other two rat studies were performed by the same research group and investigated testicular effects on adult rats exposed to glyphosate technical at doses of 0, 2, or 50 mg/kg/day (Liu et al. 2022a; 2022b). The first study collected blood, testes, and intestine samples from the rats after two months (Liu et al. 2022a). The exposed rats had impaired testis architectural structure, reduced sperm motility, and increased sperm malformation ratio. The authors hypothesized that these effects might have been linked to changes in gut microbiota. In the second study, blood, testes, and other reproductive parameters were collected after four months (Liu et al. 2022b). Exposed rats had decreased sperm quality and quantity, disruptions in the blood-testis barrier, testicular oxidative stress, changes in the regulation of certain testicular genes, and upregulation of an estrogen receptor. For some of these effects, a dose-dependent relationship was observed.
    - A meta-analysis investigated the impacts of glyphosate on multiple reproductive hormones in rats (e.g., testosterone, follicle-stimulating hormone, and estradiol) (Mohammadi et al. 2022). Criteria for inclusion were rat studies with a reported mean hormone concentration in treatment and control groups and with clear dosing units. Among 284 candidate studies, eight met the inclusion criteria, and these studies all investigated effects in male rats. The authors reported an overall statistically significant effect between glyphosate exposure and decreases in multiple hormones, but the study did not investigate effects separately for glyphosate technical exposure and GBF exposure.
- *Findings in female rats.* Three publications reported on reproductive effects in female rats associated with exposures to glyphosate technical or GBFs:
    - The first study dosed pregnant rats with glyphosate technical or GBF (Roundup, MAGNUM SUPER II, Argentina) at 2 mg/kg/day from gestational day nine until weaning (Lorenz et al. 2020). Various parameters were measured in female pups until post-natal day 90, at which point they were mated. Blood and uterine samples were collected at gestational day five and reproductive parameters were measured at gestational day nineteen (e.g., pregnancy rates, implantation sites, resorption sites). When compared to the control group, the female pups of rats treated with both glyphosate technical and GBF had higher rates of preimplantation loss, 17β-estradiol serum

levels, and ERα protein expression when compared to the control group. There was no difference in other measured parameters, such as progesterone levels or ERα protein transcription levels.

- The second study investigated the effects of a high oral dose (350 mg/kg/day) of GBF (Roundup, MAGNUM SUPER II, Argentina) from gestational day nine through the end of weaning on post-natal day 21 (Lorenz et al. 2019). Female offspring were mated on post-natal day 90, and uterine samples were collected at gestational day five. For GBF-treated rats, results indicated upregulation in the expression of ER-α mRNA of uterine tissue, potentially caused by various epigenetic changes.
- The third study investigated reproductive effects of two pesticides (Ingaramo et al. 2019). Female pups were dosed with 2 mg/kg/day of GBF via subcutaneous injection; other doses were used to investigate endosulfan and a mixture of the two pesticides. Dosing occurred on post-natal days one, three, five, and seven. Uterine samples were collected from one group of female pups sacrificed on day eight. A second group of rats was mated on post-natal day 90, and reproductive parameters were measured on gestational day nineteen. In pups, the GBF-treated group showed an increase in luminal epithelial hyperplasia and increased expression of progesterone and Hoxa10 receptors. In the adults, the GBF group (and the mixed pesticide exposure group) showed increases in post-implantation losses.

- *Findings in male mice.* One study investigated reproductive effects of glyphosate technical and GBF in male mice (Pham et al. 2019). Pregnant mice were orally dosed with 0.5, 5, or 50 mg/kg/day of GBF (Roundup 3 Plus, France) via drinking water from gestational day ten to twenty days post-partum. Young mice were sacrificed, and tissue and blood samples were collected at the ages of five days, twenty days, 35 days, and eight months. Significant differences were observed between certain treatment groups and the control group for the following measures: testis morphology in twenty-day-old mice, serum testosterone in 35-day-old mice, and spermatozoa number in the two lowest-dose groups. While significant associations were found in pair-wise group comparisons, results did not show a consistent dose response in any individual effect. The authors conclude that GBFs "could cause endocrine-disrupting effects on male reproduction."
- *Findings in female mice.* Three publications reported on reproductive effects in female mice associated with exposures to glyphosate technical. This research did not consider exposure to GBFs:
  - The first study investigated ovarian effects in mice orally dosed with five exposure levels of glyphosate technical (0.25, 0.5, 1, 1.5, or 2 mg/kg). Dosing occurred five days a week for twenty weeks (Ganesan and Keating 2020). Blood and organs were collected in the proestrus phase of the estrous cycle. Results showed that ovarian weight and follicle number increased only at the highest dose. Some changes to protein abundance were also observed. The authors acknowledged that "whether these protein changes translate to alterations in protein function within the ovary remains unclear." There was no impact on estrous cyclicity, other organ weights, and circulating levels of 17β-estradiol and progesterone.
  - The second study investigated the ovarian effects on mice orally dosed with glyphosate technical at 2 mg/kg/day for five days a week for five weeks or for ten weeks (Ganesan et al. 2020). Blood and organs were collected in the proestrus phase of the estrous cycle. This study observed no statistical differences between treatment and control groups for the various endpoints studied (i.e., organ weights, estrous cyclicity, ovarian follicle numbers, abundance of ovarian mRNA encoding genes).
  - The third study investigated ovarian effects in female mice exposed to 2 mg/kg/day of glyphosate technical for ten weeks, at which point they were mated (Novbatova et al. 2022). Reproductive parameters, blood, and tissue samples were collected at gestational day one, gestational day fourteen, and one week after weaning. The authors reported that glyphosate technical exposure reduced pregnancy success (from 75 percent to 55 percent), reduced ovarian weight in the post-weaning group, and decreased secondary follicle number in the post-weaning group. Glyphosate was also associated with changes in some ovarian and hepatic proteins.

- *Findings in other animals.* Three studies investigated the effects of glyphosate technical and GBF on the reproductive system of guinea pigs, pigs, and sheep:
    - A study investigated the effects of a GBF on the male reproductive system of guinea pigs (Mutwedu et al. 2021). The animals were orally dosed with 0, 186, 280 or 560 mg/kg/day of GBF (WILLOSATE, Democratic Republic of Congo) for 60 days, at which point the animals were sacrificed and blood and organs were collected. Compared to the control groups, all treatment groups had significant decreases in testicular weight, sperm motility, and sperm viability. Treatment groups also had increases in sperm abnormalities and changes in various blood parameters.
    - Researchers investigated the effects of a GBF on the female reproductive system of 28-day old, weaned piglets (Fu et al. 2021). The animals were orally fed a diet with GBF (Roundup, US) concentrations of 0, 10, 20 and 40 mg/kg for 35 days. The day after dosing ended, animals were sacrificed, blood samples were collected, and reproductive measurements were taken. Results showed that GBF affected the morphology of the uterus and ovary and was associated with increasing luteinizing hormone-releasing hormone, increasing gonadotropin-releasing hormone, increasing testosterone, and decreasing follicle-stimulating hormone.
    - A study investigated the effects of GBF on the female reproductive system of sheep (Alarcón et al. 2019). Prepubertal lambs were orally or subcutaneously administered 2 mg/kg/day of GBF (Roundup Full II, Argentina) for fourteen post-natal days. On post-natal days fifteen and 45, blood samples were collected and analyzed for glyphosate and AMPA; ovaries and uteri were collected on post-natal day 45. When compared to non-exposed controls, exposed animals' ovaries had altered follicular dynamics, increased proliferation of granulosa and theca cells, and decreased mRNA expression of different hormones. The uteri had decreased cell proliferation. These effects were observed in the group that had GBF orally administered and in the group that had GBF subcutaneously administered.

Review of recent in vitro articles (2019-2023) on reproductive toxicity. ERG's literature search identified thirteen additional recent *in vitro* studies investigating indicators of reproductive toxicity associated with glyphosate or GBF exposure. One of the studies was conducted in the United States; the others were conducted in other countries. A study that investigated potential treatments to mitigate impacts of glyphosate exposure is not discussed here (Bhardwaj et al. 2022). As noted previously, the *in vitro* studies provide insights into the potential for a substance to have a toxic effect, but these studies are not predictive of actual effects because responses observed in isolated cells might not reflect what occurs in entire organisms, because the exposure environment in larger organisms differs from that of cells in a laboratory setting, and because the *in vitro* studies often need to use exposure concentrations high enough to observe and characterize effects (and these concentrations might be considerably higher than what humans are exposed to environmentally or through their diets).

Seven *in vitro* studies investigated glyphosate or GBF treatment-related effects on male reproductive parameters (e.g., serotonin cell function and sperm quality). For research involving glyphosate technical, positive findings were reported for impacts on blood-testes barrier integrity (Gorga et al. 2020; Antonine et al. 2022), sperm quality (Nerozzi et al. 2020), and mitochondrial respiration efficiency of sperm cells (Ferramosca et al. 2021). In contrast, three studies found no effects of glyphosate on sperm quality at all doses tested (Spinaci et al. 2022; Torres-Badia et al. 2021; Torres-Badia et al. 2022). In the studies that tested glyphosate technical along with GBF containing POEA, the effects observed for the GBF/POEA formulations were greater than those for glyphosate technical (e.g., Nerozzi et al. 2020) or the glyphosate technical resulted in no observed effects (e.g., Torres-Badia et al. 2022).

Five *in vitro* studies investigated the effects of glyphosate technical or GBFs on cell lines relevant to the female reproductive system. For research involving glyphosate technical, positive findings were reported for impacts on prostaglandins and protein levels in the cervix (Wrobel et al. 2022), oocyte developmental competence (Spinaci et al. 2020), reactive oxide species in oocytes (Zhiqiang et al. 2022; Zhang et al. 2019b; Yahfoufi et al. 2020), and altered mRNA and mitochondrial membrane potential in oocytes (Zhiqiang et al. 2022; Zhang et al. 2019b). As was the case with male reproductive effects, studies that tested both glyphosate technical and GBF (including POEA) showed greater effects in the GBFs than in glyphosate technical.

2.5.6 Neurotoxic Effects

Information on glyphosate neurotoxicity is based on findings from major scientific assessments and on 30 recent (2019-2023) peer-reviewed publications identified in ERG's literature search.

Review of findings from major scientific assessments. EPA's 2017 draft human health risk assessment for glyphosate reviewed findings from two *in vivo* toxicity studies conducted in rats (EPA 2017b). One was an acute study of doses up to 2,000 mg/kg/day. The other was a sub-chronic (90 day) study with doses up to 1,630.6 mg/kg/day. In both studies, which were conducted according to the EPA neurotoxicity screening battery, no adverse effects were observed, and therefore no LOAEL was reported. EPA concluded that "there was no evidence that glyphosate is neurotoxic."

ATSDR's *Toxicological Profile for Glyphosate* summarized evidence of glyphosate neurotoxicity in humans and in laboratory animals (ATSDR 2020). For humans, ATSDR considered findings from three epidemiological studies published between 2007 and 2018. One AHS publication found that exposure to GBFs was not associated with the incidence or prevalence of Parkinson's disease (Kamel et al. 2007). A cross-sectional study of farmers in China found no associations between GBF exposure and abnormalities of peripheral nerve conduction (Zhang et al. 2018). A third study conducted in Washington found a positive association between increased odds of premature mortality from Parkinson's disease and living within 1,000 meters of farms where GBFs were assumed to have been applied (based on crop type) (Caballero et al. 2018). ATSDR also describes a case study of a 38-year-old man who developed Parkinson's symptoms four years after ingestion of 200 ml of glyphosate (Eriguchi et al. 2019).

For animals, ATSDR reviewed five *in vivo* studies that examined the neurotoxic effects following glyphosate technical exposures and eight *in vivo* studies that examined neurotoxic effects following GBF exposures. The studies all considered oral exposure to different species of mice and rats. ATSDR cites studies of glyphosate technical that found changes in neurotransmitter levels in a dose-dependent manner starting at doses of 75 mg/kg/day. ATSDR summarizes another study that reported changes in a "marker of synaptic terminals in the hippocampus" at an exposure dose of 5 mg/kg/day, but the underlying research publication noted that "a direct effect of glyphosate alone or its formulation on the central nervous system is currently not clear" (Dechartres et al. 2019). ATSDR's review concludes that neurological endpoints "do not appear to be particular targets of glyphosate toxicity."

EFSA's 2015 assessment likely considered many of the same underlying publications that factored into EPA's 2017 draft human health risk assessment. Based on the *in vivo* studies considering glyphosate technical exposure, EFSA concluded that "no potential for neurotoxicity or immunotoxicity was detected in glyphosate-administered rats" (EFSA 2015).

Four other assessments commented on the neurotoxicity of glyphosate. First, the FAO/WHO joint meeting stated that there was "no evidence of neurotoxicity" for glyphosate technical in an acute and a 90-day rat study (FAO/WHO 2016). Second, USFS discusses neurotoxicity findings in EPA's 1993 reregistration decision for glyphosate and reviews several *in vivo* and *in vitro* studies of glyphosate technical and GBFs published up until 2010; however, the USFS report does not include a summary statement on evidence for glyphosate neurotoxicity (USFS 2011). Third, FSCJ reviewed a study in rats that considered three different glyphosate technical grades and concluded that glyphosate "had no neurotoxicity" (FSCJ 2016). Finally, AVCMA's assessment found that "the weight- and strength-of-evidence demonstrate that glyphosate is not genotoxic, carcinogenic, or neurotoxic" (APVMA 2016).

Review of recent (2019-2023) peer-reviewed studies examining neurotoxicity in humans. ERG's literature search identified seven recent peer-reviewed publications that evaluated glyphosate neurotoxicity in humans: two epidemiological studies and five review articles. An overview of the epidemiological studies follows:

- A case-control study of 38,331 singleton births in an agricultural region of California examined the relationship between autism spectrum disorder (ASD) in children and local pesticide use (von Ehrenstein et al. 2019). Exposure to eleven pesticides, including some containing glyphosate, was estimated from the quantity applied to crops within 2,000 meters of residential birth addresses during the developmental period, which this study considered to be the three months before pregnancy, pregnancy, and the first year of life. Exposure was characterized as "any" or "none." Based on this measure, local use of six

pesticides (including glyphosate) was significantly associated with ASD both when considering potential exposures throughout pregnancy and during the first year of life. When adjusting for other pesticides, models for glyphosate and one other pesticide remained significant for certain exposure metrics and in subset analyses with cases of ASD with intellectual disability. While the authors made conclusions about glyphosate, GBFs were presumably the pesticide applied in the agricultural region that was researched.

- A cross-sectional study of 288 farmers from Uganda investigated the association between pesticide exposures and neurobehavioral outcomes (Fuhrimann et al. 2021). Approximately an equal number of traditional farmers and organic farmers were interviewed on their sociodemographic characteristics, medical history, work history, personal protective equipment (PPE) use, and pesticide use. The questionnaire asked about 53 different pesticides and a quantitative estimate of exposure over the past year was calculated based on self-reported responses. The farmers were also asked to complete eleven neurobehavioral tests. Results showed that 77 percent of participants used glyphosate, and a statistically significant association was observed between estimated glyphosate exposure and impaired visual memory. No other individual pesticide was associated with outcomes. As with the previous study, while the authors made conclusions about glyphosate, GBFs were presumably the pesticide applied in the areas that were researched.

Among the review articles, one conducted a systematic literature review of the epidemiological literature as of December 2021 and assessed quality following EPA guidelines (Chang et al. 2023). Results from this search identified 25 articles, five of which were "high-quality" and eight were "moderate-quality." The high-quality studies investigated associations with Parkinson's disease (two AHS studies), depression, (two AHS studies), and peripheral nerve conduction (one Chinese study). All high-quality studies resulted in null findings. Mixed results were observed among the "moderate-quality" studies.

In contrast, two review articles reported finding sufficient evidence of associations between GBFs and a broad range of neurological endpoints (Madani and Carpenter 2022; Costas-Ferreira et al. 2022). The first of these reviews claims that GBFs "have significant adverse effects on the brain and behavior and increase the risk of at least some serious neurological diseases" (Madani and Carpenter 2022). This review also identifies effects on the gut microbiome as a potential mechanism for a broad range of neurological endpoints. The second review systematically identified studies of glyphosate and GBF in humans, animals, and *in vitro* (Costas-Ferreira et al. 2022). Even though most of the cited studies were conducted on GBFs, the authors conclude that it is "unequivocal that exposure to glyphosate produces important alterations in the structure and function of the nervous system of humans."

Two other reviews broadly discuss the link between pesticides in general and ASD (He et al. 2022a; Ongono et al. 2020).

Review of recent literature (2019-2023) on neurotoxicity in laboratory animals. ERG's literature search identified eighteen recent citations for publications that involved evaluating neurological effects associated with exposures to glyphosate or GBFs in animals. These included seven *in vivo* mice studies, five *in vivo* rat studies, two letters to the editor, three review articles, and one publication that ERG was not able to obtain (hence that article is not considered further).

Seven studies investigated neurotoxic effects of glyphosate technical, GBFs, or both in mice. Their findings, along with insights from two reply articles, follow. The first study listed was conducted by researchers in the United States and considered glyphosate technical exposures. The others were conducted by researchers in Argentina, Brazil, Japan, and Morocco and considered GBF exposures.

- Male and female mice aged four months were orally dosed with glyphosate technical at concentrations of 125, 250, or 500 mg/kg/day for fourteen days, at which point urine, plasma, and brain samples were collected (Winstone et al. 2022). A statistically significant dose-response was observed between treatment levels and levels of glyphosate and AMPA in the brain and glyphosate in urine. Glyphosate exposure was also associated with higher levels of the pro-inflammatory cytokine, TNFα, in both plasma and the brain.

- Male mice were orally dosed with GBF (Roundup, USA) at concentrations of 250 mg/kg/day or 500 mg/kg/say for acute (one day), sub-chronic (six weeks), and chronic (twelve weeks) durations (Bali et al. 2019). For each group, on the last day of exposure, a battery of tests was administered to the mice. Mice from the sub-chronic and chronic groups were sacrificed to determine levels of acetylcholinesterase (AChE) and selected antioxidant enzymes. Mice at both exposure levels in the sub-chronic and chronic groups had recognition and retention memory impairments. The working memory of chronically exposed group was also affected. Results also showed significant decreases in AChE and antioxidant enzymes in different parts of the brain.
- Female mice were orally dosed with GBF (Roundup, USA) at levels of 250 mg/kg/day or 500 mg/kg/day from the first day of pregnancy to postnatal day 21 (Ait-Bali et al. 2020). Sensory-motor skills were evaluated in juvenile pups (postnatal day five to 25) and adult pup behavior was monitored (day 60). Adult tissue samples were also collected following behavioral tests. Results showed that GBF was associated with changes in maternal behavior, delay in innate reflexes and deficit in motor development in juvenile offspring, and various changes in adult offspring behavior (e.g., decrease of locomotor activity, sociability, leaning). Tissue samples also showed neurochemical and molecular changes to parts of the brain.
- Male mice were dosed with GBF (Glifloglex, Argentina) via intranasal administration three times per week for four weeks at an estimated dose of 50 mg/kg/day of glyphosate (Gallegos et al. 2020). A functional observational battery was administered 15 days after the final dose, and brain tissue samples were collected. The glyphosate-dosed mice did not have impaired functional parameters but did have impaired oxidative stress markers in the brain. In addition, the treatment group had various changes in cholinergic and glutamatergic pathways (e.g., reduced number of cholinergic neurons, changes in expression of certain receptors).
- Pregnant female mice were orally administered GBF (Roundup) via drinking water at a concentration of 0.075 percent weight per volume (a dose was not estimated) from pregnancy, through lactation, and until adulthood (Del Castilo et al. 2022). Behavioral tests were conducted on pups on postnatal day 60 and 80. Brain and gut tissue samples were collected after behavioral tests. Results showed that the GBF-treated mice had certain behavior changes (impaired social behavior and increased repetitive behavior), no changes in cognitive function, increased levels of phagocytic cells in parts of the brain, and morphological and functional changes in the gut including changes in the microbiome.
- During pregnancy and lactation, mice were orally administered a one percent (weight per volume) drinking water solution of GBF (Roundup Maxload, Japan), which translates into a dose of approximately 50 mg/kg/day of glyphosate (Pu et al. 2020). Behavioral tests were performed on juvenile mice (postnatal day 28 to 35), and blood and brain samples were collected. Results showed "autism spectrum disorder (ASD)-like" behavioral changes and enzymatic changes to the brain that the authors indicate are linked to ASD. Results of gut tissue analyses show changes including abnormal composition of microbiota. In a letter in response to Pu et al. (2020), Reeves and Dunn (2021) take issue with the conclusion that glyphosate is the cause of the observed changes in the offspring. They note that the observed changes in maternal behavior and the significant decreases in maternal body weight in the GBF-treated group could have caused the observed changes in offspring, which therefore might not be direct glyphosate-related effects. They also note that the GBF studied contains harmful additives that could have caused some of the observed changes. Two of the authors from the original research article, Hashimoto and Hammock (2021), responded; they noted that other research has shown similar effects in mice treated with pure glyphosate.
- The purpose of the final study in mice was to demonstrate the protective effects of quercetin therapy following various behavioral changes in GBF-treated mice (Bicca et al. 2021).

Five studies investigated neurotoxic effects of glyphosate technical, GBF, or both in rats. The studies were conducted by researchers in Brazil, France, Japan, and Sweden. Every study considered GBF exposures, and one also considered glyphosate technical exposures.

- Pregnant rats were orally administered GBF (Roundup G, Sweden) via drinking water at a concentration of three percent from pregnancy through postnatal day fifteen (Cattani et al. 2021). The estimated glyphosate dose for this study was 70 mg/kg/day. Male offspring were sacrificed on postnatal day 90 and brain tissue samples were collected and analyzed for neuropeptide changes. Results showed that the GBF-treated group had significant changes in the substantia nigra and hippocampus areas of the brain. The authors concluded that GBF "may perturb critical neurodevelopmental processes."
- Pregnant rats were orally administered GBF (ZappQI620, Brazil) at a dose of 50 mg/kg/day from pregnancy through postnatal day 22 (De Oliveira et al. 2022). Behavior was observed in dams (postnatal days 2 to 6) and offspring (days 5, 13, and 28 to 32). Brain samples were collected from offspring after behavioral tests. In the GBF-treated group, changes were observed in the behavior of dams (e.g., total distance traveled, percentage of exploration) and offspring (e.g., early social communication, olfactory discrimination, social play behavior, and the exploration of objects). Oxidative stress was also observed in the brains of offspring in the GBF-treated group.
- Pregnant rats were orally administered GBF (Roundup Transorb, Brazil) at 5 or 50 mg/kg/day from gestational day eighteen until postnatal day five (De Souza et al. 2019). Serum and brain samples were collected from male offspring on postnatal day 90. GBF-treated rats had significant changes in the expression of genes related to antioxidants and inflammation in parts of the brain and significant increases in serum markers of neurodegenerative diseases (lysophosphatidylcholine and phosphatidylcholine).
- Pregnant rats were orally administered GBF (Roundup, USA) at 5 mg/kg/day from gestational day 10 through postpartum day 22 (Dechartres et al. 2019). Postpartum maternal behavior was observed, feces were collected (days 9, 19, and 22), and tissue samples (serum and brain) were collected (day 22). GBF-treated dams had significant differences in licking behavior towards pups, maturation of certain neurons in parts of the brain, expression of certain proteins in parts of the brain, and composition of gut microbiota.
- Pregnant rats were orally administered glyphosate or GBFs from gestational day six to postnatal day 21 (Ojiro et al. 2023). Three dosing scenarios were used: glyphosate technical at a concentration of 1.5 percent in the diet, glyphosate technical at a concentration of 3 percent in the diet, and GBF (Turnout Liquid Agent, Japan) at a concentration of 1 percent in drinking water. The exposure scenarios had doses ranging from 250 mg/kg/day to 4,650 mg/kg/day. Various measures of neurogenesis were collected in juvenile and adult offspring. In offspring, both glyphosate and GBF treatments altered body weight and had a range of neurotoxic effects, such as changes in neural cell proliferation, numbers of certain granule cells, and regulation of antioxidant genes. The publication noted that some effects in adult rats were "more evident" in the groups exposed to GBFs when compared to the groups exposed to glyphosate technical. The authors concluded that this difference suggested that surfactants in the GBF might have contributed to the increased neurotoxicity of the formulations.

Three review articles synthesized information from *in vivo* studies and other lines of evidence on glyphosate neurotoxicity. The first review article conducted a systematic literature review of *in vivo* mammalian studies as of June 2021 (Moser et al. 2022). Results from this search identified 27 articles, eleven of which were considered "unreliable." Seven acceptable studies considered glyphosate technical. The review concludes that the evidence does "not demonstrate a consistent impact of glyphosate on the structure or function of the mammalian nervous system." The second review article focuses on gut microbiota and the potential downstream effects of neurological disorders (Rueda-Ruzafa et al. 2019). This review, which does not describe the literature search and review protocol, discusses the potential mechanism and evidence for glyphosate-induced dysbiosis—an imbalance between beneficial and harmful bacteria in the gut—which can lead to neurotoxic outcomes. The authors conclude that "more studies are required." The third review article broadly describes the relationship between pesticides and neurogenesis (Rossetti et al. 2020).

Review of recent in vitro articles (2019-2023) on neurotoxicity. ERG's literature search identified five recent *in vitro* studies investigating indicators of neurotoxicity associated with glyphosate or GBF exposure. One was conducted in the United States, and the others in other countries. For research involving glyphosate technical, positive findings were reported for impacts to expression neuronal genes (Martinez et al. 2020; Masood et al. 2021),

dendritic complexity (Luna et al. 2021), and synaptic spine formation and maturation (Luna et al. 2021). Impairments in the differentiation potential of human neuroepithelial stem cells were seen with GBF exposure but not with glyphosate technical (Reis et al. 2022). GBF was also associated with effects on the blood-brain barrier permeability (Martinez et al. 2019).

2.5.7 Endocrine Effects

Information on glyphosate's endocrine effects is based on findings from major scientific assessments and on 21 recent (2019-2023) peer-reviewed publications identified in ERG's literature search. Some studies that focused on other health endpoints and that are described in other sub-sections of this report also review endocrine effects.

Review of findings from major scientific assessments. EPA's Endocrine Disruptor Screening Program (EDSP) applies a consistent methodology to evaluate the endocrine disruption potential of toxic substances. EDSP reviews existing studies and conducts its own to investigate whether chemicals interfere with the estrogen, androgen, and thyroid systems. If so, EDSP characterizes potential effects and dose-response behavior. In its 2015 EDSP assessment, EPA found "no convincing evidence of potential interaction with the estrogen, androgen or thyroid pathways" for glyphosate (EPA 2015b).

ATSDR's *Toxicological Profile for Glyphosate* summarized evidence of glyphosate endocrine disruption in humans and laboratory animals (ATSDR 2020). For humans, ATSDR reviewed three epidemiological studies—two from the AHS—that investigated relationships between GBF usage and thyroid diseases or function. The first AHS study did not find an association between various thyroid diseases and ever-use of glyphosate (Goldner et al. 2010). The second AHS study, with more than double the number of participants (N=35,150), found an association between self-reported hypothyroid disease and ever-use of glyphosate (Shrestha et al. 2018). The study also found this association for ten other pesticides. Among participants with the most intense pesticide use, the hypothyroidism risk was greatest for five pesticides other than glyphosate, and it was not significantly increased for glyphosate. The third study was a cross-sectional study of farmers in Thailand (Kongtip et al. 2019). A review of the third study is provided below.

For animals, ATSDR described the underlying animal studies that EPA considered in its review of endocrine disruption potential, which focused entirely on glyphosate technical. ATSDR also reviewed several *in vivo* studies reported in the literature, which primarily considered GBFs. Those GBF studies had mixed findings on certain endpoints (e.g., intratesticular testosterone levels), and the effects identified for GBFs primarily pertained to changes in hormone levels, gene expression, and protein expression.

EFSA's 2015 risk assessment report noted that the information available at the time indicated that "endocrine disrupting properties are not met" for glyphosate, but the report also indicated that the complete set of EPA EDSP testing results were not available for evaluation (EFSA 2015). In 2017, after the EDSP results were final, EFSA published a follow-up analysis specific to glyphosate's endocrine disruption potential. This analysis found that "the weight of evidence indicates that glyphosate does not have endocrine disrupting properties through estrogen, androgen, thyroid or steroidogenesis mode of action based on a comprehensive database available in the toxicology area" (EFSA 2017).

Three other assessments commented on glyphosate's endocrine disruption potential. The FAO/WHO joint meeting concluded that evidence from many validated *in vivo* and *in vitro* assays "clearly demonstrate that there is no interaction with estrogen or androgen receptor pathways or thyroid pathways" (FAO/WHO 2016). Similarly, the APVMA assessment, completed in the same year, concluded that "there is no scientific justification for classifying glyphosate as an endocrine disrupter *[sic]*" (APVMA 2016). The USFS assessment summarizes multiple *in vitro* studies related to endocrine disruption, but the underlying research considered GBFs that are used in other countries and reportedly not used in the United States (USFS 2011). USFS did not issue an endocrine disruption conclusion based on the data that were available at the time.

Review of recent (2019-2023) peer-reviewed studies examining endocrine effects in humans. ERG's literature search identified one recent epidemiological study and two review articles related to glyphosate's endocrine disruption potential.

Kongtip et al. (2019) conducted a cross-sectional study of 222 organic farmers and 195 conventional farmers in Thailand that examined the relationship between pesticide use and thyroid hormone levels. Blood samples from all participants were analyzed for five thyroid hormones, and pesticide exposure was estimated from self-reported usage accounts. For glyphosate, no significant relationships were observed between the exposure metric (i.e., the amount of glyphosate-containing pesticide applied) and blood concentrations of four hormones—free triiodothyronine (FT3), thyroxine (FT4), T3, and thyroid-stimulating hormone (TSH); a marginal increase in T4 was significantly associated with the glyphosate exposure metric. Significant associations were also reported for multiple combinations of other pesticides and one or more thyroid hormones. The significance of this study's glyphosate finding is unclear because the study relied on self-reported pesticide usage estimates, the participants were exposed to multiple pesticides, and the two study populations had statistically significant differences in multiple characteristics known or suspected to be associated with thyroid function, including age, sex, smoking history, and years of pesticide use.

One review article summarizes evidence for the effects of glyphosate and GBFs on thyroid function (Romano et al. 2021). The review compiles a range of observations from epidemiological studies (primarily those identified earlier in this section) and *in vivo* studies in mammals and non-mammalian species. It reaches several conclusions, including that the relationship between glyphosate and GBF exposure to thyroid outcomes "is still a very controversial subject," and further research is needed to better understand this matter.

Another review article (Muñoz et al. 2021) summarized research published through 2020 and evaluated whether glyphosate exhibits ten factors recently reported in the literature as being characteristic of an endocrine disruptor. The authors conclude that glyphosate "behaves like an endocrine disruptor" and notes that prospective epidemiological studies in humans are needed to confirm this finding. Although a considerable portion of the evidence cited in this review is based on exposures to GBFs, a conclusion is drawn for glyphosate.

Review of recent literature (2019-2023) on endocrine effects in laboratory animals. Eleven recent publications present endocrine findings from *in vivo* studies that dosed animals with either glyphosate technical, GBFs, or both. These include seven publications describing *in vivo* rat studies, one publication of an *in vivo* mouse study, and three review articles.

The *in vivo* studies that investigated endocrine parameters in rats involved exposure to glyphosate technical, GBFs, or both. Three were conducted by researchers in Argentina, two by researchers in Nigeria, and the others by researchers in Brazil and Tunisia. All but one of the studies focused exclusively on GBFs, not glyphosate technical. The study that considered glyphosate technical is summarized first, below.

- One study investigated the effects of both glyphosate technical and GBF on various blood parameters in rats (Owagboriaye et al. 2019). Male albino rats were orally dosed with glyphosate technical or GBF (Roundup Original, USA) at 3.6, 50.4, or 248.4 mg/kg/day for twelve weeks, at which point blood was collected. Results showed a dose-dependent relationship between GBF exposure and increasing levels of stress hormones (corticosterone, aldosterone), decreasing levels of antioxidants (glutathione, catalase), decreasing enzyme activity (catalase, butyrylcholinesterase), and increasing lipid peroxidation. However, for glyphosate technical, no dose-dependent relationships were observed.
- Two studies from the same research group investigated the effect of GBF (Magnum Super II, Argentina) exposure on male rat mammary gland development (Gomez et al. 2019; 2020). In both studies, pregnant rats were dosed with 0, 3.5, or 350 mg/kg/day of GBF from gestational day nine until weaning. Across the two studies, serum and mammary gland samples were collected from animals at some combination of days 21 and 90. Positive findings included less developed mammary glands in the highest exposure groups, reduced estrogen receptor alpha (ERα) expression, epigenetic changes to ERα, and decreases in the mRNA expression of various hormones proteins (e.g., ERα, Ccnd1, IGF1R, and EGFR) involved in mammary gland development; however, the studies reported no difference in mammary gland development at day 21, no changes in blood hormone levels at days 21 and 90, and no structural changes in the mammary gland.
- A study that investigated the effects of GBF (Roundup FULL II, Argentina) on female rat mammary gland development injected pups with 2 mg/kg/day of GBF on postnatal days one, three, five, and seven (Zanardi et al. 2020). Serum and mammary gland samples were collected after twenty months of age.

Results showed that GBF-exposed rats had morphological changes (higher rates of hyperplastic ducts and a fibroblastic-like stroma) in the mammary glands and higher expression of steroid hormone receptors.

- A study investigated the effects of GBF (Kalach 360 SL, Tunisia) on hormonal changes and other outcomes (Hamdaoui et al. 2020). Female rats were treated by gavage with 0, 126, or 315 mg/kg/day of GBF for 60 days, at which point plasma, thyroid glands, and other samples were collected. Results showed treatment groups had morphological changes in the thyroid gland, decreased FT3 and FT4, and increased TSH. A dose-dependent relationship was observed for hormonal changes.
- A study investigated the effects of GBF (Roundup Transforb, Brazil) on various thyroid and other outcomes (Costa Reis et al. 2021). Pregnant rats were treated with gavage GBF in water from gestational day eighteen to postnatal day five at doses of 5 and 50 mg/kg/day. Male pups were weaned at postnatal day 21 and sacrificed on day 90, at which point cerebellums were collected. Results showed that GBF-treated rats had altered mRNA levels of thyroid hormone receptors and other intergenerational changes in thyroid hormone regulators, including epigenetic changes.
- One study was designed to investigate whether the effects of high-fructose corn syrup on diabetes symptomatology was mediated by the presence of GBF (ForceUP, China) (Kale et al. 2021). This study is not summarized further because it did not focus on direct effects of GBF.

One *in vivo* study investigated the effects of glyphosate technical on the endocrine system of mice (Zhao et al. 2021). Male mice were orally dosed with 0.5% glyphosate technical via drinking water for four weeks. Mice were sacrificed and blood and tissue samples were collected at four weeks or twelve days after that. Results showed that serum testosterone was reduced in mice treated with glyphosate and that specific testosterone related mRNA and protein levels in testes were reduced.

One review article, authored by an employee of a glyphosate registrant, evaluates the potential for glyphosate to interact with the estrogen, androgen, and thyroid pathways by reviewing and analyzing data from EPA's EDSP, additional guideline studies, and recent literature studies (Levine et al. 2020). The article describes the literature search methodology, including the databases searched, the search strings used, and the relevancy criteria applied. The review describes the lines of evidence across each hormonal pathway and concludes that "glyphosate does not have endocrine-disrupting properties through estrogen, androgen, thyroid and steroidogenic modes of action" and that "exposure to glyphosate will not result in adverse effects through an endocrine mechanism."

Another review article summarizes journal articles relevant to endocrine effects and attempts to explain apparent inconsistencies in the published literature (de Araujo-Ramos et al. 2021). The article indicates that it considered "some of the studies on glyphosate reproductive and endocrine toxicity," but it does not describe how the articles were selected. The article attributes some apparent inconsistencies to the testing of glyphosate technical versus the testing of GBFs. It also comments on the limitations of current regulatory testing strategies (e.g., they do not consider all potential mechanisms of endocrine disruption). Finally, the article acknowledges that apparent inconsistencies can result from differences in study design and execution.

A third review article summarizes the evidence for glyphosate-related endocrine disruption, with a focus on female reproductive effects associated with environmentally relevant exposures (Ingaramo et al. 2020). The article reviews *in vitro* and *in vivo* studies, but it does not describe the approach to identifying relevant literature. The authors identify two potential mechanisms of endocrine disruption on the estrogen pathway: the action of glyphosate aromatase (the enzyme responsible for biosynthesis of estrogens) and the activation of an estrogen response element, such as upregulation of the ERα gene. The authors conclude that glyphosate and GBFs "may have the properties to be EDCs [endocrine-disrupting chemicals]" and that the endocrine disruption can contribute to adverse reproductive effects in females, and they acknowledge that "much research is still needed to know the real toxicity effects of glyphosate and GBHs in humans."

Review of recent in vitro articles (2019-2023) on reproductive toxicity. Seven additional recent studies involved *in vitro* testing of glyphosate, GBFs, or both to assess indicators of endocrine disruption. The studies were authored by researchers from China, Italy, Brazil, Chile, and the United Kingdom. Most of these publications considered exposures to glyphosate technical, not GBFs.

Two *in vitro* studies investigated effects on thyroid cells. The first found the highest doses of glyphosate technical to be associated with reduced cell viability and proliferation, increased production of reactive oxygen species, and changes in mRNA levels for some thyroid related genes (Coperchini et al. 2023). The second found that low doses of GBF tested (Roundup, Brazil) were associated with both increased cell death and increased cell proliferation (Dal'Bo et al. 2022).

Two *in vitro* studies investigated the effects of glyphosate technical on changes in testosterone production. The first study found that glyphosate inhibited the secretion of testosterone in treated cells in a dose-dependent manner and that higher exposure changed the levels of various proteins involved in testosterone formation (Xia et al. 2020). The second study, conducted in the same cell line as the previous study, showed that mitochondrial malfunction may cause the changes in protein levels that contributed to decreased testosterone synthesis (Lu et al. 2022).

Two *in vitro* studies investigated the effects of glyphosate technical on changes in estrogen production. The first study found that glyphosate produces estrogen-like effects, alters estrogen receptor α (ERα), and increases cell proliferation in a breast cancer cell line (Muñoz et al. 2023). The second study tested three pesticides and found that only glyphosate inhibited aromatase, which is an enzyme required for the biosynthesis of estrogen; however, glyphosate was the only pesticide to not show direct estrogen-like activity (Zhang et al. 2020).

One *in vitro* study investigated the effects of six pesticides on adipocytes and found that glyphosate did not induce adipogenesis (Biserni et al. 2019).

2.5.8 Developmental Effects

Information on glyphosate developmental toxicity is based on findings from major scientific assessments and on fifteen recent (2019-2023) peer-reviewed publications identified in ERG's literature search. These publications document effects associated with exposures prior to conception, *in utero*, or during early-life. The publications that investigated both developmental endpoints and other health endpoints are reviewed here and in other sections of this report, as appropriate.

Review of findings from major scientific assessments. In its 2017 draft human health risk assessment for glyphosate, EPA's hazard characterization conclusions were based entirely on *in vivo* toxicology studies. In rats, EPA reports "developmental toxicity was seen at or above the limit dose (1,000 mg/kg/day)." That is the highest dose evaluated in some of the animal studies. In rabbits, "developmental toxicity (decreased fetal weight) was seen only at high doses"; however, maternal toxicity occurred at lower doses (EPA 2017b). The lowest reported developmental LOAEL across the studies EPA reviewed was 1,000 mg/kg/day, based on decreased fetal weight in rats, and the lowest developmental NOAEL was 300 mg/kg/day from a study of rabbits. (Note, the point of departure used in EPA's evaluation of dietary exposure scenarios is based on a developmental study; however, the effect of concern is maternal toxicity, not developmental effects in offspring.)

ATSDR's *Toxicological Profile for Glyphosate* summarized evidence of glyphosate developmental toxicity in humans and in laboratory animals (ATSDR 2020). For humans, ATSDR considered findings from seven epidemiological studies published between 1997 and 2018 that had mixed results for developmental outcomes. These studies all considered exposures to GBFs, not to glyphosate technical.

A retrospective cohort study from Canada reported a positive association between the glyphosate exposure metric (i.e., use of GBFs on farms during pregnancy) and an increased risk of miscarriage (Arbuckle et al. 2001). ERG notes that positive associations were reported for multiple groups of herbicides, and the study did not quantify glyphosate exposures—it only considered whether glyphosate was ever applied in fields where the participants lived. A cross-sectional study among Minnesota farm families reported a positive association between "ever use of glyphosate" and attention-deficit/hyperactivity disorder in children born in the area (Garry et al. 2002). ERG notes that this study also did not quantify glyphosate exposures, relied on applicators' recollections of past pesticide use, and only considered self-reported health outcomes. On the other hand, the other epidemiological studies found no associations between indicators of parental exposures to glyphosate due to pesticide use and multiple developmental outcomes, including miscarriages, preterm delivery, small for gestational age, congenital malformations, low birth weight, neural tube defects, and head circumference (Savitz et al. 1997; Garcia et al. 1998; Rull et al. 2006; Sathyanarayana et al. 2010; Parvez et al. 2018). Given the mixed results and limitations

across the epidemiological studies, ATSDR concluded that "these results were not considered sufficient for drawing conclusions on the risk of development toxicity associated with glyphosate exposure in humans."

For animals, ATSDR reviewed ten studies that examined developmental effects following glyphosate technical exposure and nine studies that examined developmental effects following GBF exposures. The studies all considered oral exposure that occurred during gestation or after birth, and they considered exposures to different species of mice, rats, and rabbits. ATSDR summarized the glyphosate technical studies by noting developmental toxicity was observed at doses (LOAEL = 1,234 mg/kg/day) that also caused maternal toxicity. ATSDR summarized the GBF studies by listing various adverse effects (e.g., testicular lesions, decreased sperm production, increased fetal skeletal malformations) observed in rats at lower doses (5 to 500 mg/kg/day).

The 2015 EFSA assessment documents various developmental effects reported in rats and rabbits (EFSA 2015). One issue not acknowledged in the EPA and ATSDR reports was the finding of cardiac malformations in one rabbit study; however, the majority of EFSA peer reviewers did not classify the finding as developmental toxicity because severe maternal toxicity occurred at the same exposure dose and because other developmental studies in rabbits did not replicate the finding. Note that EFSA's acceptable daily intake and acute reference dose for glyphosate are based on a developmental study in rabbits, but the derivation is based on the NOAEL for maternal toxicity, not on developmental toxicity in offspring.

The FAO/WHO joint meeting also noted potential developmental effects in three of seven rabbit studies (FAO/WHO 2016). As with the EFSA assessment, certain high-dose fetal effects in rabbits, such as cardiac malformation and absent kidney, were deemed secondary to maternal toxicity. The assessment reported NOAELs and LOAELs consistent with those noted above, and it reported that glyphosate is not teratogenic.

The USFS glyphosate risk assessment's findings are based on a similar profile of *in vivo* laboratory animal studies (USFS 2011). The assessment makes some important observations related to developmental toxicity not addressed in the other assessments. For example, USFS reviewed two research publications that suggest a low potential for glyphosate to transfer across the human placenta; one of the studies estimated that fifteen percent of "maternal circulation" of glyphosate crossed the placenta to "fetal circulation" (Mose et al. 2008). USFS ultimately concluded that "there is no indication that technical grade glyphosate causes birth defects." Other statements in this risk assessment pertaining to developmental effects are based on an animal study that EPA has since reported as having spurious results (see Section 2.5.9 for further details); those statements are not summarized here.

ERG also reviewed the assessments issued by authorities in Australia (APVMA 2016), Canada (PMRA 2017), and Japan (FSCJ 2016). These assessments inferred comparable LOAELs and NOAELs (where reported) and presented findings generally consistent with those summarized above. The Australian assessment summarized an expert review that was commissioned specifically to investigate several assertions that had been made about glyphosate's toxicity, including that it causes birth defects. The expert review concluded, among other things, that "glyphosate is extremely unlikely to cause reproductive or developmental toxicity in humans under normal conditions of exposure" (APVMA 2016).

Review of recent (2019-2023) peer-reviewed studies examining developmental toxicity in humans. ERG's literature search identified six peer-reviewed publications that evaluated glyphosate developmental toxicity in humans.

- A prospective cohort study of 187 high-risk pregnant women in Indiana found that glyphosate was detected in the urine of almost all (99 percent) participants; the median specific-gravity-adjusted maternal urinary concentration was 3.08 ng/mL (Gerona et al. 2022). The urine samples were collected during prenatal care visits, and measured glyphosate levels were negatively associated with birth weight percentile. The association remained significant in regression analyses adjusted for demographic, geographic, and health characteristics; gestational age; and drug use. Statistically significant relationships were not observed between glyphosate urine levels and neonatal intensive care unit admission in main analyses, though some associations were observed in subset analyses.
- A pilot study of 94 mother-infant pairs in the U.S. evaluated the association between urinary concentrations of glyphosate and its metabolite (AMPA) and anogenital distance (Lesseur et al. 2021). Among the 45 female and 49 male participants, two anogenital distance measures were collected after birth. Urinary glyphosate and AMPA collected in the second trimester were detected in 95 percent and 93

percent of the urine samples at median concentrations of 0.22 ng/mL and 0.14 ng/mL, respectively. After adjusting for covariates, longer ano-fourchette distance in females and urinary AMPA (but not urinary glyphosate) were significantly associated. The relevance of this finding to glyphosate is unclear, because AMPA is a degradation product of multiple substances (not just glyphosate) and can be formed in the environment or in the body.

- A cohort study of 163 pregnant women in the U.S. evaluated the association between urinary concentrations of glyphosate and AMPA and length of gestation (Lesseur et al. 2022). The study consisted of 69 preterm births (<37 weeks) and 94 term births randomly selected from the same cohort. Glyphosate and AMPA were detected in 94 percent of maternal urine samples collected during the second trimester, and the median specific-gravity-adjusted maternal urinary concentration was 0.22 ng/mL. Urinary glyphosate levels were not significantly associated with preterm birth or gestational length in univariate analyses. After adjusting for maternal age, race/ethnicity, and education level, a significant relationship was observed between urinary concentrations of both glyphosate and AMPA and shortened gestational length in the subset of 90 participants who had spontaneous births.
- A case-control study of 304,906 live, singleton births in North Carolina examined the relationship between ten birth defects and pesticide exposures (Rappazzo et al. 2019). Exposures to seven different pesticides, including GBFs, were estimated based on the quantity of pesticide applied to crops within 500 meters of the participants' residences within one month before conception to three months of pregnancy. When compared to unexposed controls, the individuals with higher estimated glyphosate exposure had more birth defects of various types. Some associations were only observed in the highest exposure group (90th percentile). These associations, however, were also observed for almost every other pesticide that was evaluated; in many cases, the reported associations for glyphosate were no longer significant when adjusted for other pesticides that were highly correlated with GBFs (e.g., pesticides containing cyhalothrin and mepiquat).
- A nested case-control study assessed the association between maternal glyphosate and AMPA urine levels in a pregnancy cohort in Puerto Rico (Silver et al. 2021). Urine samples were collected from 53 cases (women with preterm pregnancies) and 194 randomly selected controls (women with full-term pregnancies) during the first and third prenatal visit (approximately weeks eighteen and 26). On the third prenatal visit, glyphosate and AMPA were detected in the urine of 79.3 percent and 52.4 percent of participants, respectively; the median maternal specific-gravity-adjusted urinary glyphosate concentration for the entire cohort was 0.5 ng/mL. Statistically significant differences in both glyphosate and AMPA urinary concentrations at visit three were observed between cases and controls in unadjusted models. The authors reported a significant association between both urinary glyphosate and AMPA concentrations measured around the 26th week of pregnancy and increased odds of preterm birth.
- A case-control study of children under five years of age in Thailand evaluated associations between the risk of developmental delay and pesticide use by the mother (Juntarawijit et al. 2021). The participants included 442 children with diagnosed suspected developmental delay (as measured by a series of cognitive tests) and 413 controls with normal development. Cases and controls were matched for age, gender, and place of residence. No statistically significant increases in risks for development delay in children were observed among the individuals who reported "ever use" of a pesticide that contained glyphosate.

Review of recent literature (2019-2023) on developmental toxicity in laboratory animals. Nine recent *in vivo* studies involved dosing animals with glyphosate, GBFs, or both to assess indicators of developmental toxicity. Four studies were conducted on rats, three on mice, one on sheep, and one on pigs. All nine studies were conducted by researchers outside the U.S.

Two studies investigated the effects of glyphosate technical in rats. In one study, pregnant rats were dosed with glyphosate technical via intraperitoneal injection at 24 or 35 mg/kg, and offspring were evaluated for neurobehavioral effects (Coullery et al. 2020). Dose-dependent relationships were observed for changes in reflexes development, motor activity, and cognitive function, though statistical significance was only observed in the higher exposed group. The authors noted that gestational exposure to glyphosate was associated with an inhibited neuronal signaling pathway (Wnt5a-CaMKII) that could contribute to developmental neurotoxicity.

A second study investigated the effects of glyphosate on the development and endocrine systems in rats (Manservisi et al. 2019). Groups of pregnant female rats were dosed with glyphosate technical and a GBF (Roundup Bioflow) via water at 1.75 mg/kg/day through 120 days after birth. Offspring were sacrificed and examined at either six or thirteen weeks after birth. Anogenital distance was significantly increased in males treated with glyphosate technical and in both males and females treated with GBF. Increases in plasma thyroid stimulating hormone were observed in the six-week glyphosate-treated group but not in the thirteen-week group. Additional effects (e.g., delay in age at first estrous, increases in serum testosterone in females, and decrease in plasma 5α-dihydrotestosterone in males) were statistically significant in the GBF-treated group, but not in the glyphosate technical treated group.

Two additional rat studies examined associations between GBF exposure and developmental effects of the uterus. In the two similarly designed studies from the same institute in Argentina, 2 mg/kg/day of a GBF was injected into the nape of pups on post-natal days one, three, five, and seven (Ingaramo et al. 2022; Schimpf et al. 2022). In both studies, alterations to uterine development were observed across a range of endpoints. The alterations included hyperplasia and epigenetic alterations on day eight, altered estrous cyclicity on day 120, and leiomyomas and pre-neoplastic glandular lesions on day 600.

Three studies from Brazil investigated the effects of GBF (Roundup Original D, Brazil) in drinking water on mice (Barbosa et al. 2022; Gomes et al. 2022; Teleken et al. 2020). In all three studies, pregnant mice from the same lineage were dosed with 0.5% of GBF (equivalent to 1.85 mg/ml of glyphosate) in drinking water. The concentration was selected based on groundwater contamination levels observed in agricultural settings in the region. Dosing occurred throughout pregnancy and for 30 days of lactation. The researchers reported this exposure scenario being equivalent to a dose of 420 mg/kg/day of GBF. In the first study, muscle tissues were collected from mice sacrificed at 150 days of life (Barbosa et al. 2022). Many effects were reported for the exposed mice, including reduced body weights, reduced nasoanal length, reduced proportion of muscle fibers and number of nuclei, increased weight of specific muscles and connective tissues, and increased adiposity. In the second study, multiple insulin sensitivity and glucose sensitivity tests were administered to the pups (Gomes et al. 2022). Body parameters in GBF-treated mice were unaffected, but glucose tolerance was increased on post-natal day 60 and insulin tolerance at day 143. This study also found indications of liver inflammation. The third study examined various effects in both dams and pups (Teleken et al. 2020). The dams showed no changes in reproductive parameters, physical characteristics of offspring, or biochemical metabolic parameters; however, pups in the treatment groups showed differences in various male reproductive parameters: delayed testis descent, reduced sperm number in the cauda epididymis, increased intratesticular testosterone content, and increased plasma luteinizing hormone and a related protein.

Researchers in Argentina investigated the effects of GBF on mammary gland development in sheep (Altamirano et al. 2023). Ewe were dosed with 2 mg/kg/day of GBF (Roundup FULL II, Argentina) via subcutaneous injection in the nape or orally from post-natal day one through day fourteen. Lambs from both GBF groups had larger mammary glands and smaller terminal duct lobular units. Decreased mRNA expression of various receptors and proteins were observed in the insulin-like growth factor 1 (IGF-1) signaling system.

One other study conducted on pigs investigated protective effects of the natural extract, betaine, on placental toxicity (Bai et al. 2022). This study is not summarized further.

Review of recent (2019-2023) in vitro studies related to developmental toxicity. ERG's literature search did not identify peer-reviewed publications issued since 2019 that evaluated glyphosate developmental toxicity *in vitro*. However, some *in vitro* studies described in the endocrine effects and reproductive effects sections have implications for developmental endpoints.

2.5.9 Renal Effects

Information on glyphosate renal toxicity is based on findings from major scientific assessments and on ten recent (2019-2023) peer-reviewed publications, which included one review article.

Review of findings from major scientific assessments. In its 2017 draft human health risk assessment for glyphosate, EPA's hazard characterization conclusion reports "minor indicators of toxicity to the eyes, liver, and/or kidney" (EPA 2017b). This conclusion was based on EPA's review of fourteen chronic *in vivo* studies of rats and

mice that EPA found acceptable for evaluating glyphosate toxicity. The risk assessment summarizes four studies that reported renal toxicity in both mice and rats. Examples of observed effects include interstitial nephritis, proximal tubule epithelial basophilia and hypertrophy, and kidney papillary necrosis. Excluding one study that EPA determined had spurious results, the lowest reported LOAEL for these studies' renal effects was 1,214 mg/kg/day, and the lowest NOAEL for these two studies was 361 mg/kg/day.

ATSDR's *Toxicological Profile for Glyphosate* summarized evidence of glyphosate renal toxicity in humans and in laboratory animals (ATSDR 2020). For humans, ATSDR reviewed a case-control study of farmers from Sri Lanka with chronic kidney disease of unknown etiology (CKDu) (Jayasumana et al. 2015). The study has notable limitations, including limited exposure characterization and potential confounding. Further, the relevance of this study to the U.S. population is unclear, given differences in pesticide application procedures between the U.S. and Sri Lanka. ATSDR also reviewed two case reports of GBF poisoning incidents that resulted in acute kidney injury in one individual and acute kidney failure in the other (Ozaki et al. 2017; Picetti et al. 2018), and a retrospective cohort study of GBF poisoning incidents, which also found evidence of acute kidney injury (Cho et al. 2019). The exposures during these poisonings, however, are considerably higher than what the general population experiences.

For animals, ATSDR separately summarized laboratory studies with glyphosate technical and GBF exposures. For glyphosate technical, the literature documented evidence of renal toxicity, but in studies with extremely high exposure levels or dosing strategies (intraperitoneal administration) of limited relevance to human exposures. For GBFs, the animal studies had mixed results for renal toxicity, and ATSDR concluded that "there is some uncertainty regarding the role of glyphosate in the reported effects" (ATSDR 2020).

EFSA's hazard characterization report from 2015 only mentions renal toxicity in the context of a dosing study of ruminants (goats and cattle) which EFSA notes "may be more sensitive than monogastric animals" (EFSA 2015). In the study, animals dosed with glyphosate at 1,000 mg/kg/day exhibited various adverse effects, including evidence for renal toxicity. The relevance of this observation to humans is not known given the cross-species physiological differences in digestive systems.

The FAO/WHO joint meeting also noted potential effects on kidneys. The high-level summary concluded that in four of seven studies, depending on the statistical approach, relatively high dietary doses (e.g., >4,841 mg/kg) of glyphosate led to kidney adenomas in male mice (FAO/WHO 2016). The meeting noted the "increases were marginal and occurred at the highest dose only and that other studies that used appreciably higher doses did not find any excess." One of these studies also identified a NOAEL of 814 mg/kg/day and LOAEL of 4,841 mg/kg/day partially based on proximal tubular epithelial basophilia.

The USFS assessment notes that kidney toxicity in studies of glyphosate technical "have not been reported consistently and are not severe" (USFS 2011).

Other assessments listed in Table 2 did not address renal toxicity.

Review of recent (2019-2023) peer-reviewed studies examining renal toxicity in humans. ERG's literature search identified five peer-reviewed publications that evaluated glyphosate renal toxicity in humans. One of these studies was conducted in the U.S. and the remaining four considered populations in Nicaragua, Sri Lanka, and Thailand.

The U.S. study used a cross-sectional design to assess connections between glyphosate exposure and kidney function in infants and young children (Trasande et al. 2020). The study considered 108 children with ages ranging from less than 30 days to eight years. A urine sample was collected from each participant, and samples were analyzed for glyphosate concentration and three biomarkers of kidney injury. While the study detected glyphosate in the urine of twelve participants, the detected concentrations were not associated with kidney injury biomarkers. The authors noted that the small sample size was a study limitation and recommended further study with larger cohorts.

The studies outside the U.S found:

- A case-control study evaluated evidence for CKDu among a cohort of 350 young adults at risk for Mesoamerican nephropathy in rural Nicaragua (Smpokou et al. 2019). The researchers collected urine samples from participants before and after the local sugarcane harvest and analyzed the samples for

concentrations of 26 toxicants, which included glyphosate, other pesticides, pesticide metabolites, mycotoxins, metals, and metalloids. Glyphosate was detected in approximately one-third of the samples, but none of the toxicants was found to be associated with impaired renal function.

- Another study evaluated biomarkers of kidney function and urine concentrations of two pesticides in 210 Sri Lankan farmers with more than ten years of farming experience (Abdul et al. 2021). The authors noted that urinary levels of glyphosate and paraquat are "potentially linked to the subsequent decline in kidney function" observed among some participants, but other factors (e.g., significant demographic and lifestyle differences among the regions studied) might have contributed to the observed effect. The magnitude of pesticide exposures in Sri Lankan farmers might not be representative of conditions in the U.S. The authors acknowledge, for example, that "excessive use of pesticides above the recommended usage" was common among participants; that a considerable portion of the farmers did not use PPE when applying pesticides; and that many farmers did not follow recommended approaches for mixing, storing, and disposing of pesticides.
- A third study considered 59 farmers in northern Thailand who have been actively using pesticides for at least one year (Mueangkhiao et al. 2020). The participants answered questions about their pesticide use, medical history, and other risk factors for kidney disease; each participant provided a blood sample and a urine sample that were tested for multiple kidney function biomarkers. Of the six biomarkers studied, only increased serum creatinine levels were significantly associated with self-reported use of pesticides that contain glyphosate. The significance of the increased serum creatinine levels is unclear, as the highest levels observed in the study were within the range of reference values in healthy individuals reported in the paper, and the authors acknowledged that participants "had normal ranges of kidney biomarkers." Study limitations included small sample size and incomplete characterization of pesticide exposure.

The only other recent publication pertaining to renal toxicity in humans is a review article that synthesized and interpreted evidence for glyphosate exposure as a contributing factor to CKDu (Gunatilake et al. 2019). The article acknowledges multiple risk factors for CKDu, including dehydration from heat stroke, poor nutrition, and exposure to various toxic substances (e.g., metals, cyanobacteria, mycotoxins); it also identifies exposures to pesticides, particularly glyphosate and paraquat, as "likely compounding factors" and possibly "primary factors" for CKDu. The authors hypothesize that CDKu results from "synergistic toxicity of glyphosate in combination with a number of different toxic agents, including paraquat, excessive fluoride and phosphate exposure, heavy metals, surfactants and pathogenic toxins, along with dehydration." Though supporting evidence for this hypothesis is presented, the authors conclude by noting that further research is warranted to understand the roles of various factors in causing CKDu.

Review of recent literature (2019-2023) on renal toxicity in laboratory animals. Three recent *in vivo* studies involved dosing animals with GBFs (Roundup) and assessing indicators of kidney toxicity. The first study investigated renal effects in 28 piglets with dietary exposure to Roundup for 35 days (Qiu et al. 2022). A dose-response relationship was observed between four exposure concentrations (corresponding to doses of 0 to 40 mg/kg/day of glyphosate) and certain plasma biomarkers of kidney damage (cystatin-C [Cys-C] and NGAL). The study identified significant increases in the mRNA levels of certain transcription factors that have been linked to kidney disease. Some significant differences in antioxidant levels were also observed in the highest treatment group.

The purpose of the two other studies was to demonstrate the protective effects of other chemicals on the toxic effects of Roundup exposure. One study demonstrated how administration of linseed oil can ameliorate Roundup-related oxidative stress in the liver and kidney of male rats (Djaber et al. 2020). The second study evaluated how administration of melatonin ameliorates glyphosate-related renal toxicity (Ding et al. 2022). These studies are not summarized further because their primary purpose was not to establish dose-response relationships for Roundup toxicity.

Review of recent literature (2019-2023) on in vitro studies related to renal toxicity. Two additional recent publications were reviewed. First, an *in vitro* study involved dosing a human renal proximal tubule cell line (HK-2) with glyphosate technical for 24 hours at four concentrations (0 to 60 µM) (Gao et al. 2019). A dose-dependent relationship was observed with increasing cell viability and oxidative stress. Second, a review article summarized

molecular mechanisms relating environmental exposures to CKDu (Upamalika et al. 2022). While multiple stressors besides glyphosate are discussed (e.g., heat, dehydration, exposures to heavy metals), the review attributes caspase activation and lipid peroxidation as a potential mechanism by which glyphosate causes renal toxicity. Neither study reported findings directly observed in animals or humans.

2.5.10 Other Human Health Effects

Information on glyphosate toxicity on other health endpoints is described here based on findings from major scientific assessments and on recent (2019-2023) peer-reviewed publications identified in ERG's literature search. These health endpoints are presented here (as opposed to in their own sections) because there were fewer than ten recent articles identified in the literature search that were categorized as primarily affecting these endpoints.

Gastrointestinal Effects

Across multiple assessments, gastrointestinal effects were identified as the most sensitive endpoint.

In EPA's draft human health risk assessment, the chronic reference dose (cRfD) and short and intermediate term oral levels of concern (LOC) were based on LOAELs (175 mg/kg/day) and NOAELs (100 mg/kg/day) derived from gastrointestinal effects—diarrhea, few feces, and no feces (EPA 2017b). EPA notes that "this endpoint is not typically considered to be an adverse effect per se." However, a dose-dependent relationship was observed for this endpoint in both a rat and a rabbit study. EPA noted that the chosen LOAEL is "protective of all of the other effects and durations in the database."

EFSA's conclusions from its previous health assessment list the gastrointestinal tract as one of many "main target organs" of glyphosate (EFSA 2015). The ADI, AOEL, and ARfD derived by EFSA are all based on a NOAEL (50 mg/kg/day) from a developmental rabbit study that found gastrointestinal signs. However, other, more serious, effects were also observed in this study, such as increased incidences of post-implantation losses. In the minutes of the most recent peer review meeting for EFSA's current assessment, gastrointestinal irritation in a rabbit study is mentioned again as being the basis of a NOAEL to derive the ARfD, but in this case the NOAEL is set at 150 mg/kg/day (EFSA 2022).

ATSDR's *Toxicological Profile for Glyphosate* lists gastrointestinal effects as the most sensitive endpoint for deriving acute (LOAEL of 175 mg/kg/day), intermediate (LOAEL of 350 mg/kg/day), and chronic (457 mg/kg/day) MRLs based on the same studies reviewed by EPA (ATSDR 2020). ATSDR also describes numerous case reports that describe gastrointestinal effects following ingestion of GBFs.

ERG's literature search identified six recent (2019-2023) *in vivo* studies that evaluated gastrointestinal effects associated with exposures to glyphosate and GBFs. Two studies in rats found that both glyphosate and GBF were associated with changes in the gut microbiome, including a reduction in certain beneficial bacteria, and in the case of GBF, increases in the number of certain fungi (Mesnage et al. 2021c; 2022a).

The remaining four studies investigated the effects of intestinal morphology. Mice dams were given drinking water with 0.5% GBF and the male offspring, which were exposed only through lactation, were found to have altered morphology of the intestinal wall (Panza et al. 2021). Rats were administered GBF orally and via inhalation at three exposure levels; those in the highest exposure group had dysplastic lesions in the esophagus and small and large intestine (de Maria Serra et al. 2021). One study found that weaned piglets dosed with 0, 10, 20, or 40 mg/kg/day of GBF (Roundup, U.S.) for 35 days did not have changes in intestinal morphology, but they did have increases in oxidative parameters in the duodenum and various changes in mRNA expression (Qiu et al. 2020). In another study on suckling piglets, a high dose of GBF (100 mg/kg/day) was associated with damaged intestinal morphology and barrier function (Bai et al. 2023) among other findings.

Respiratory Effects

The agency assessments presented limited information on respiratory effects associated with exposure to glyphosate technical and GBFs. For example, EPA's draft health risk assessment only mentions the respiratory effects of glyphosate in the context of acute incidents; EFSA's assessment does not address respiratory effects.

ATSDR's *Toxicological Profile for Glyphosate* identifies fifteen publications investigating the association between respiratory effects in humans and GBF exposure. Nine of these publications were associated with the AHS and investigated various outcomes (e.g., rhinitis, wheezing, asthma, chronic bronchitis) (Hoppin et al. 2002; 2006a;

2006b; 2007; 2008; 2009; 2017; Slager et al. 2009; 2010). Many of the results from these studies were null except in subset analyses. ATSDR notes that "many of these studies did not account for the use of other pesticides." Finally, ATSDR describes a study that found an association between respiratory illness in medical record data and aerial spraying of GBFs in Colombia (Camacho and Mejia 2017). The remaining human studies summarized by ATSDR relate to the intentional ingestion of glyphosate.

ERG's literature search identified three recent (2019-2023) publications that evaluated respiratory effects associated with exposures to glyphosate and GBFs, including one epidemiological study and two *in vivo* studies. One study found significant decreases in measures of breathing function (e.g., forced expiratory volume) compared to the day before in Thai farmers one day after the application of GBF (Sidthilaw et al. 2022). The purpose of the two *in vivo* studies was to investigate how glyphosate modifies lung inflammation following endotoxin (lipopolysaccaride) exposure (Pandher et al. 2021a; 2021b).

Immunological Effects

The agency assessments presented limited information on immunotoxicity of glyphosate technical and GBFs. EPA's draft human health risk assessment concluded that "there was no evidence that glyphosate is neurotoxic or immunotoxic" (EPA 2017b). ATSDR's Toxico*logical Profile for Glyphosate* did not identify any human studies on immunotoxicity and concluded that immunological endpoints "have been evaluated, but do not appear to be a particular target of glyphosate toxicity" (ATSDR 2020). EFSA concluded that "no potential for neurotoxicity or immunotoxicity was detected in glyphosate-administered rats" (EFSA 2015).

ERG's literature search identified seven recent (2019-2023) publications that evaluated immunotoxic effects associated with exposures to glyphosate and GBFs, including two review articles, four *in vivo* studies, and one *in vitro* study. One review article identified three epidemiological studies that found associations between GBF exposures and respiratory symptoms (Maddalon et al. 2021). The three studies identified in the Maddalon et al. (2021) review, two of which were a part of the AHS, found some associations between exposure to many different pesticides and rhinitis or wheeze episodes (Chatzi et al. 2007; Slager et al. 2009; Hoppin et al. 2017). A separate review article broadly describes the evidence for immunotoxic effects and concludes that in humans, glyphosate "causes inflammation, and affects lymphocyte functions and the interactions between microorganisms and the immune system" (Peillex et al. 2020).

One *in vivo* study on rat dams orally administered glyphosate technical at 0.5 mg/kg/day found reduced immune response in the lungs of offspring (Buchenauer et al. 2022). Another study found a greater neuro-inflammatory response in the pups of rat dams exposed to water with 5 mg/L of GBF or AMPA compared to rats exposed to glyphosate technical (Duque-Diaz et al. 2022). A study on mice dosed 250 or 500 mg/kg/day of GBF for 28 days found significantly decreased immune cells in the blood (i.e., decreased leukocyte, neutrophil, lymphocyte, and monocyte counts) in the high-dose group (He et al. 2022). A study on dairy cows found that glyphosate exposure through feed (0.0012, 0.1126, 0.1328 mg/kg/day) did not impact immune cell parameters in blood, such as white blood cell counts, T-cell subpopulations, leukocytes (Schnabel et al. 2020). The one *in vitro* study found cytotoxic effects on human immune cells (Barbasz et al. 2020).

Cardiovascular Effects

The agency assessments presented limited information on cardiovascular effects of glyphosate technical and GBFs. EPA's and EFSA's risk assessment did not address this endpoint (EPA 2017b; EFSA 2015). ATSDR's *Toxicological Profile for Glyphosate* describes two AHS publications that found no association between use of GBFs and myocardial infarctions (Dayton et al. 2010; Mills et al. 2009) and many case reports of high dose exposures adversely impacting the cardiovascular system. No animal studies were identified that examined the cardiovascular system and no conclusions are drawn for this endpoint.

ERG's literature search identified two recent (2019-2023) publications that evaluated cardiovascular effects associated with exposures to glyphosate and GBFs including one *in vivo* study and one *in vitro* study. A study on rats sub-chronically exposed to GBF (Roundup Original DI, Brazil) via oral dosing or inhalation observed a higher incidence of fatty streaks in the aorta for both routes of exposure (Maia et al. 2021). An *in vitro* study conducted on an isolated guinea pig heart found that GBF (Roundup), but not glyphosate technical, impacted heart contractility and induced arrhythmias (Printemps et al. 2022).

Hepatic Effects

The agency assessments presented limited information on hepatic effects of glyphosate technical and GBFs. EPA's draft human health risk assessment notes "minor indicators of toxicity to the liver" observed at high doses (EPA 2017b). EFSA's conclusions from its previous health assessment lists the liver as one of many "main target organs" of glyphosate in rodents (EFSA 2015); however, adverse liver effects are generally described in studies at high doses or with other target endpoints. Minutes from EFSA's most recent peer-review meeting note liver lesions, along with many other endpoints, at a dose of 595.2 mg/kg/day in a two-year rat study (EFSA 2022).

ATSDR's *Toxicological Profile for Glyphosate* identifies one nested case-control study that found higher levels of urinary glyphosate, urinary AMPA, and glyphosate residue in patients with nonalcoholic steatohepatitis compared to controls with nonalcoholic fatty liver disease. This study observed a dose-dependent relationship between extent of GBF exposure (as gauged by urinary glyphosate and AMPA) and advanced stages of fibrosis (Mills et al. 2020). ATSDR also describes multiple rodent studies with hepatic effects (e.g., increased liver weight) at high doses above 1,678 mg/kg/day (ATSDR 2020).

ERG's literature search identified seven recent (2019-2023) publications that evaluated hepatic effects associated with exposures to glyphosate and GBFs including one epidemiological study, five *in vivo* studies, and one *in vitro* study. The one epidemiological study (Mills et al. 2020) was considered in ATSDR's Toxicological Profile and is described above. One study that gestationally exposed mice to glyphosate technical or GBF (0.5% solution, dose not reported) found that, compared to controls, mice in both glyphosate and GBF treatment groups had changes in hepatic lipid levels (e.g., elevated total cholesterol, LDD cholesterol) and the expression of corresponding genes (Ren et al. 2019). Another study found that rats dosed at 0.5, 50, or 175 mg glyphosate/kg/day had signs of hepatic steatosis (fatty liver disease) following GBF treatment but not following glyphosate technical treatment (Mesnage et al. 2022b). A third study found that GBF-exposed female rats had liver damage as indicated by markers of steatosis (Hamdaoui et al. 2019). The two other *in vivo* studies on non-rodent mammals found minimal effects. One found that a GBF had "no toxic effects on the liver of dairy cows" (Heymann et al. 2021). The other found that piglets fed a diet containing GBF caused varied liver enzyme activity indicating some "damage and dysfunction," but that the liver "self-alleviated" (Fu et al. 2020). The *in vitro* study focused on the protective effects of antioxidants on a human liver cell line exposed to a GBF (Endirlik et al. 2022).

Case Reports

Sixteen publications described case reports of acute glyphosate toxicity events in humans. Many case reports describe patients presenting with a range of manifestations, including kidney failure (Kimura et al. 2020; 2022), gastrointestinal damage (Hao et al. 2020; Luo et al. 2019; Tao et al. 2022), multi-organ failure (Bigner et al. 2021; Wang et al. 2019), cardiovascular failure (Ghosh et al. 2021), respiratory failure (Kunapareddy and Kalisetty 2021), encephalopathy (Yokoyama et al. 2021), Parkinson's disease (Eriguchi et al. 2019), coma (Takeuchi et al. 2019), and refractory hypotension (Lee and Min 2019). Another case-report focused on calculating the rate of change of glyphosate in urine and blood following a poisoning event (Cho et al. 2019).

A review study identified all case reports received by French Poison Control Centers and found that poisonings with tallow amine-containing GBFs were more severe than poisonings with non-tallow amine-containing GBFs (Langrand et al. 2020). Another review article identified biomarkers of acute kidney injury stages following GBF poisoning incidents (Wijerathna et al. 2020).

Other Noncancer

ERG's literature search identified 21 publications that were not categorized into the sections described previously.

Five publications describe glyphosate's effects on other health endpoints. One study identified changes in 27 metabolites in the blood of workers from three factories in China and identified four that could be used as a biomarker for glyphosate exposure (Zhang et al. 2022). Another identified specific genetic polymorphisms in factory workers in China that were associated with lower serum cholinesterase indicating a potential genetic biomarker of susceptibility (Cai et al. 2020). The remaining studies considered whether glyphosate (and other GBF ingredients) should be classified as "skin sensitizers" (Lindberg et al. 2020), researched evidence for oxidative stress biomarkers among exposed individuals (Salazar-Flores et al. 2020), and evaluated protective effects of other compounds in addressing effects attributed to glyphosate exposure (Nozdrenko et al. 2021; Turkmen et al. 2019).

Eleven publications describe cytotoxic effects of glyphosate technical and GBFs across a range of tissue types, including intestinal cells (Bai et al. 2022), liver cells (Conte et al. 2022; Costa et al. 2022; Hao et al. 2019, Mehtiyev et al. 2023; Silva et al. 2022), breast cells (Coppola et al. 2022), nerve cells (Hao et al. 2019; Neto da Silva et al. 2020), blood cells (Kwiatkowska et al. 2020), colon cells (Truzzi et al. 2021; Silva et al. 2022), fibroblasts (Truzzi et al. 2021), and lung cells (Hao et al. 2019).

Four review articles covered a broad range of human health effects. One review article summarizes the *in vitro* and epidemiological literature on glyphosate and GBFs and a range of health endpoints and concludes that "it is not possible to confirm the complete safety of glyphosate" (Agostini et al. 2020). Another review article focused on the mechanisms underlying inflammation, DNA damage, and alterations in gene expression and concludes that "it is not possible to have a unequivocal opinion on the safety of glyphosate, and it appears that the human health risk associated with glyphosate could still be underestimated" (Marino et al. 2021). A third review article identified co-formulants across a range of GBFs and showed that formulations have progressively become less toxic as the specific POEA surfactant adjuvant has been replaced; original formulations contained POEA-tallow amines, which were replaced by other POEAs (ethoxylated ether amines followed by propoxylated quaternary ammonium) (Mesnage et al. 2019). A systematic review identified studies that tested both GBFs and active ingredients and found that, across multiple studies, the toxicity of certain GBFs exceeded that of glyphosate technical (Nagy et al. 2020).

2.5.11 Conclusions Regarding Human Health Effects

Below is a summary of ERG's conclusions on human health effects of glyphosate technical. For each health endpoint, we describe the overall conclusions from agency assessments and whether the recent literature presents new evidence on glyphosate technical that warrants reconsideration of agency determinations. For example, for non-cancer endpoints, we compare LOAELs described in agency assessments to LOAELs observed in more recent studies.

As mentioned previously, across assessments, gastrointestinal effects were consistently identified as the most sensitive endpoint. In its 2017 draft human health risk assessment, EPA derived a chronic reference dose (cRfD) of 1 mg/kg/day based on a study that reported a NOAEL of 100 mg/kg/day and a LOAEL of 175 mg/kg/day due to gastrointestinal effects following glyphosate technical exposure. This LOAEL was considered protective of all other observed effects across animal studies that EPA reviewed. When recent studies observe adverse effects at a lower dose (e.g., lower LOAELs), ERG provides a judgement on the overall quality of the recent evidence.

Because of the large number of GBFs, the proprietary nature of the formulations, the fact that many studies considered concentrated GBFs while actual applications often involve dilute solutions, and the fact that the scope of this scientific review focuses on glyphosate technical, conclusions are not made about the toxicity of these formulations. Even so, across a range of studies, GBFs appear to elicit a greater toxic response than glyphosate technical. Several recent journal articles specifically mention the toxicity of the surfactant adjuvant, POEA, though EPA has conducted a human health risk assessment that supported a tolerance exemption for the class of surfactants that includes POEA (EPA 2009). In one study, the toxicity of different types of GBF adjuvants were further investigated and early formulations containing POE-tallow amine were identified as the most toxic (Mesnage et al. 2019). In the European Union, POE-tallow amine has been banned as an adjuvant in GBFs (EC 2016). In the handful of studies that compare glyphosate technical, GBFs, and POEA, the effects of the latter two compounds are generally more toxic than that of glyphosate technical alone. A recent article co-authored by a representative of a glyphosate registrant found "no significant human health issues" associated with using POE-tallow amine surfactants in GBFs (Martens et al. 2019)

This section is organized around the different health endpoints evaluated in this scientific review. Before presenting that synthesis, ERG notes a few general considerations related to health effects of glyphosate:

- The mechanism of glyphosate's herbicidal action in plants—killing them by interfering with the shikimate pathway, which plants use to produce amino acids essential for growth and survival—does not apply directly to humans, because mammals do not have this pathway. However, the pathway exists in microorganisms, including those found in the human gut. Some researchers have suggested that glyphosate can indirectly cause adverse health effects by disrupting the gut microbiome. EPA reviewed

this issue in 2018 and presented several reasons why glyphosate exposure is unlikely to alter the gut microbiome in humans. These reasons include (1) the low rate of glyphosate absorption and metabolism in humans and (2) the fact that the essential amino acids impacted by the shikimate pathway are available in the human gut from other sources (e.g., the diet) (EPA 2018a). EPA noted that microorganisms in the human gut are capable of "growing and surviving" despite inhibition of the shikimate pathway (EPA 2018a). However, actual data on this issue are limited.

- ATSDR has summarized glyphosate toxicokinetics as follows: After humans ingest glyphosate, the chemical is "readily absorbed" in the human gut; it is then distributed throughout the human body in blood but does not readily accumulate in organs or tissues; very little metabolism to AMPA occurs; and any unmetabolized glyphosate is eventually excreted in urine or feces (ATSDR 2020).
- While Phase Two of this project was under way, researchers from the Centers for Disease Control and Prevention (CDC) published findings on glyphosate in the urine of U.S. residents. Specifically, as part of the 2013-2014 National Health and Nutrition Examination Survey (NHANES), CDC collected urine from a representative sample of the U.S. population over age six and detected glyphosate in 81 percent of the samples (Ospina et al. 2022). The authors noted that these results demonstrate widespread recent exposure to glyphosate in the U.S. population, likely through dietary exposure. (NHANES investigates biomarkers for other pesticides, including many that are also detected in Americans' blood or urine.) ERG did not identify any health guidelines from government agencies that directly and conclusively relate urinary glyphosate concentrations to specific adverse health effects. However, a previous publication reported on back-calculated daily doses (exposures) that would be expected to result in urinary glyphosate concentrations comparable to those recently reported by CDC; and that publication suggests that these exposure may not be of health concern (Niemann et al. 2015).
- When reviewing the evidence for toxicity of substances like glyphosate, it is important to consider the susceptibility of vulnerable populations like children. While the human epidemiological literature for glyphosate is much more extensive for adults, several publications have examined relationships between glyphosate exposure and adverse health effects in children. No associations were observed between early-life or childhood exposure to glyphosate and kidney function (Trasande et al. 2020), developmental delay (Juntarawijit et al. 2021), and biomarkers of oxidative stress (Makris et al. 2022). One study reported an association between estimated exposure to glyphosate and autism spectrum disorder, but an association was also observed for seven other pesticides (von Ehrenstein et al. 2019). Three reviews considered connections between parental pesticide exposure and cancers in their children, but the reviews did not specifically evaluate effects of glyphosate (Feulefack et al. 2021; Khan et al. 2022a; 2022b). In addition, numerous animal studies were designed to specifically explore the potential harmful effects of early-life exposure to glyphosate. These investigations involved exposures at various life-stages, such as pre-conception, *in utero*, and post-natal, to better understand health implications of glyphosate. The studies are described primarily in the developmental and reproductive sections of this report. Note that EPA considered protection of children in its recent draft human health risk assessment, specifically through review of multiple animal studies that involved glyphosate dosing during *in utero* and post-natal life stages; the Agency reported that it found "no risks of concern identified for children" for the exposure pathways that were reviewed (EPA 2018a).
- Although the epidemiological studies considered in this review offer inconsistent and sometimes conflicting results, it should be noted that establishing a clear, causal link between low-dose exposures to toxic substances (like glyphosate) and specific health outcomes is complex. Numerous variables, including individual genetic factors, behavioral choices, and simultaneous exposure to other chemicals confound these associations. The absence of a clear, causal relationship in the human data does not imply that these relationships do not exist—another possibility is that glyphosate causes harmful health effects in humans that the extensive research conducted to date has yet to characterize.

The following conclusions are meant to provide a reference for how recent epidemiological and toxicological research compares to the research that various agencies considered in their major assessments.

Cancer (see Section 2.5.4)
IARC is the only agency to list glyphosate as probably carcinogenic to humans. Across all agency assessments, NHL and MM appear to have the strongest evidence of a potential association with glyphosate; however, for various reasons (e.g., inconsistent findings across studies, application of different study selection criteria, extent to which GBF research factored into glyphosate findings), the agencies other than IARC all determined that insufficient epidemiological and animal evidence existed for any cancer type.

ERG's literature search identified 28 recent (2019-2023) studies discussing glyphosate's potential cancer effects. Some of these articles discuss the differences between the approaches and determinations from agency assessments. Several meta-analyses and pooled analyses evaluate data from studies that were already considered by the agency assessments. These analyses examined the same underlying data sets (or highly similar data sets) and reached conflicting results. Of the recent articles that discuss epidemiological data, only six present new data, but these studies did not find significant associations, or they were limited in their study designs.

The recent literature also included one article that reported *in vivo* data for glyphosate technical. Results showed that glyphosate-exposed mice, from a genetic line that can serve as an animal model for MM, developed plasma cell neoplasms, among other findings. The authors claim these results support a B-cell specific mechanism for NHL and MM; however, in this study only a single dose of glyphosate was tested (1,000 mg/L in drinking water), limiting the interpretation of results (Wang et al. 2019a).

The publications issued since the agency assessments were completed do not provide consistent evidence of a connection between glyphosate exposure and cancer. ERG infers that it is unlikely that the recent publications will cause agencies to revise their previous findings regarding glyphosate's carcinogenicity.

Reproductive Effects (see Section 2.5.5)
Across agency assessments, the lowest LOAEL for reproductive endpoints was reported by ATSDR: 640 mg/kg/day for development of abnormal sperm in male mice.

ERG's literature search identified 32 recent (2019-2013) studies discussing reproductive toxicity published since the various agencies completed their assessments. No epidemiological studies were identified, nineteen articles were identified that reported *in vivo* studies. These included several rodent studies that reported adverse reproductive effects following exposure to glyphosate technical at a dose of 2 mg/kg/day (Gorga et al. 2021; Liu et al. 2022a; 2022b; Pham et al. 2019; Lorenz et al. 2020; Novbatova et al. 2022).

For male rodents, glyphosate-dosed rats had reduced sperm quality and quantity and increased permeability of the blood-testes barrier across studies; some of these measures were dose-dependent (Gorga et al. 2021; Liu et al. 2022a; 2002b). While one of the studies found that the effects on sperm and testes of rats dosed during the juvenile stage were reversed in adulthood (Gorga et al. 2021), two studies found that the effects persisted into adulthood in the presence of continued exposure (Liu et al. 2022a; 2022b). Another study did not find a dose-dependent relationship for these same endpoints in mice (Pham et al. 2019).

For female rodents, results were mixed. Mice orally dosed with 2 mg/kg/day of glyphosate had decreased pregnancy success (from 75 percent to 55 percent) in one study, though it is unclear if this difference was significant (Novbatova et al. 2022); however, adverse effects were not observed across a range of ovarian endpoints in two other mice studies (Ganesan and Keating 2020; Ganesan et al. 2020). In a fourth study, rats dosed at 2 mg/kg/day had increased preimplantation loss, though only a single dose was tested (Lorenz et al. 2020).

The dose (2 mg/kg/day) at which effects have been reported in recent literature is two orders of magnitude lower than the LOAEL reported in the study that EPA used to derive its toxicity guidelines for glyphosate. These recent studies show that male reproductive parameters may be affected at doses considerably lower than those EPA used when deriving its chronic reference dose (cRfD), which is currently 1 mg/kg/day. The recent studies listed above were all published after EPA completed its draft human health risk assessment. However, it is unclear if the reported effects or the underlying research protocols used would qualify for consideration in agency derivation of toxicity guidelines. For instance, the research cited above does not establish a mode of action or adverse outcome pathway; and some individual studies had shortcomings (e.g., testing only one dose, lack of consistent dose-response relationships) that agencies would likely note in their upcoming assessments. The pending assessment from EFSA and the ongoing EPA assessment of glyphosate are expected to provide further context on this matter.

Neurotoxic Effects (see Section 2.5.6)
The major agency assessments primarily relied on studies submitted to EPA that utilized a standard neurotoxic battery on rats. While EPA's assessment did not identify any neurotoxic effects, ATSDR reported "less serious" neurotoxic effects, such as decreased activity, subdued behavior, and hunched posture, at the highest dose of 2,000 mg/kg/day (ATSDR 2020). This dose exceeds EPA's limit dose in animal studies for glyphosate (1,000 mg/kg/day) and it exceeds the LOAELs derived for other health endpoints considered in the agency assessments.

ERG's literature search identified 30 recent (2019-2023) journal articles that discuss glyphosate's neurotoxicity that were published since the various agencies completed their assessments. These included three epidemiological studies that considered GBF exposures, but the studies had multiple limitations associated with study design.

The recent literature also included eighteen articles reporting on *in vivo* studies, two of which evaluated glyphosate technical. One glyphosate technical study conducted on rats examined doses greater than 1,040 mg/kg/day (Ojiro et al. 2023). The second study, conducted on mice, used glyphosate technical doses between 125 and 500 mg/kg/day and found a dose-dependent increase in glyphosate and TNF-α in brain tissue, and this effect was evident at the lowest dose tested; but the study did not investigate or report frank manifestations of neurotoxicity (Winstone et al. 2022). These exposure levels are not considerably different from the LOAELs considered in the agency assessments, and it is unclear if the changes in tumor necrosis factor levels would constitute an adverse effect for assessment purposes. The remaining *in vivo* studies only considered GBFs.

A review article proposes a mechanism of action for glyphosate neurotoxicity endpoints, specifically through downstream effects on the gut and microbiome (Rueda-Ruzafa et al. 2019). Some recent *in vivo* studies provide evidence supporting this hypothesis (Del Castilo et al. 2022; Pu et al. 2020; Dechartres et al. 2019). These studies, however, were conducted with GBFs, complicating efforts to make inferences about glyphosate technical. Furthermore, EFSA's latest peer review meeting states that "studies on potential effects of glyphosate on the human and animal gut microbiome are not expected to impact the risk assessment" (EFSA 2022); and EPA, when responding to public comments on the Agency's draft human health risk assessment, made similar inferences about the likelihood of glyphosate exposures altering the gut microbiome in humans (EPA 2018a).

The publications related to neurotoxicity issued since the agency assessments indicate some biochemical changes occur in mice following exposures to 125 mg/kg/day. Because this is comparable to the LOAEL and NOAEL that EPA reported for its cRfD derivation, it is unlikely the recent publications will cause agencies to revise their previous findings regarding glyphosate's neurotoxicity.

Endocrine Effects (see Section 2.5.7)
When evaluating glyphosate-related endocrine effects, the major agency assessments primarily relied on findings from EPA's Endocrine Disruptor Screening Program, which reported "no convincing evidence of potential interaction with the estrogen, androgen or thyroid pathways" (EPA 2015b).

ERG's literature search identified 21 recent (2019-2013) journal articles that focused on glyphosate-related endocrine disruption and were issued since the agencies completed their assessments. This included one epidemiological study that reported increases in a thyroid hormone (T4) in Thai farmers exposed to GBFs and multiple other pesticides (Kongtip et al. 2019). However, the significance of this finding is unclear given the study's limitations (see Section 2.5.7).

The recent literature also documented ten *in vivo* studies, two of which evaluated glyphosate technical in rodents. A rat study that orally dosed glyphosate technical at multiple concentrations up to 248.4 mg/kg/day found no dose-dependent associations with any measured hypothalamic-pituitary-adrenal hormones and oxidative stress markers (Owagboriaye et al. 2019). Mice dosed with a single concentration of 0.5% glyphosate solution in drinking water showed reduced testosterone serum levels (Zhao et al. 2021). However, the authors did not report an estimated exposure dose for the mice, which complicates efforts to compare the findings to other studies.

Glyphosate does not appear to act directly on hormonal receptors, which would likely have been captured in traditional toxicology tests such as EPA's EDSP; but much of the recent literature describes other potential indirect mechanisms by which a chemical might affect the endocrine system (e.g., expression of hormonal proteins, receptors). This recent literature, however, is largely focused on the effects of GBF, and therefore does not directly characterize endocrine toxicity for glyphosate technical. (Note, toxic effects on the endocrine system may manifest

as toxicity on other endpoints, such as reproductive or developmental endpoints; refer to those summaries for further information.)

Because the recent publications did not report LOAELs below the values EPA used in its cRfD derivation, it is unlikely that the recent literature would alter conclusions drawn by various agency assessments regarding glyphosate's endocrine disruption properties.

Developmental Effects (see Section 2.5.8)
The major agency assessments reviewed many developmental studies in rodents and rabbits and generally observed developmental effects only at doses that also caused maternal toxicity. EPA reported a LOAEL for developmental toxicity as 1,000 mg/kg/day, exceeding the LOAELs derived from other health endpoints considered across agency assessments (EPA 2017b).

ERG's literature search identified fifteen recent (2019-2013) studies on reproductive toxicity published since the agencies completed their assessments. This included six epidemiological studies that considered GBF exposures. Mixed results were observed for associations with gestational length and preterm birth (a dichotomous variable of gestational length). First trimester urinary glyphosate levels were associated with gestational length in high-risk pregnancies (Gerona et al. 2022). However, urinary glyphosate levels during pregnancy were not associated with preterm birth in two other studies (Silver et al. 2021; Lesseur et al. 2022). Some associations were observed with AMPA measured in urine, but the implications of these findings are unclear because AMPA, though a known metabolite of glyphosate, can also form following exposures to other substances.

The recent literature also included nine articles reporting on *in vivo* studies, two of which evaluated glyphosate technical. One study found associations between glyphosate dosed via intraperitoneal injection and developmental neurotoxicity, but the route of exposure does not clearly apply to humans (Coullery et al. 2020). The only other study of glyphosate technical found that male mice pre- and post-natally treated with glyphosate technical via water at a dose of 1.75 mg/kg/day had significantly increased anogenital distance (Manservisi et al. 2019). This effect was not observed in females, and dose-response in males could not be evaluated because only a single dose was used.

The dose noted in one study of 1.75 mg/kg/day is two orders of magnitude lower than LOAELs that EPA used to derive its cRfD for glyphosate. However, results from this single study have not been replicated in other studies, and the study's limitations do not preclude the possibility that the observed effects were by chance. Considering the evidence presented in the recent literature, it is unlikely that the conclusions drawn by various agency assessments regarding glyphosate's developmental toxicity would be altered.

Renal Effects (see Section 2.5.9)
The major agency assessments evaluated many rodent studies and generally observed renal effects only at high doses that also caused toxicity in other endpoints. The LOAEL for renal effects in EPA's draft risk assessment was 1,214 mg/kg/day, exceeding the LOAELs derived from other health endpoints considered across agency assessments (EPA 2017).

ERG's literature search identified ten recent (2019-2013) studies discussing renal toxicity of glyphosate that were published since the agencies completed their assessments. These include five epidemiological studies that considered GBF exposures. One U.S. study in children detected glyphosate in 12 of 108 children, but no significant association was observed with biomarkers of kidney injury. Much of the remaining recent epidemiological literature around renal toxicity focuses on the association between pesticides in general and incidence of CKDu; and that research was conducted in other countries and regions with high CKDu prevalence (e.g., Sri Lanka, Central America). In some of this literature, statistically significant associations were observed between GBFs and CKDu. However, the potentially many unknown causes of CKDu in other countries that have different pesticide application procedures than the United States limit the generalizability of these studies.

The recent literature also included three articles reporting on *in vivo* studies; however, none of these studies evaluated glyphosate technical.

Considering the evidence presented in the recent literature, it is unlikely that the conclusions drawn by various agency assessments regarding glyphosate's renal toxicity would be altered.

Other Effects (see Section 2.5.10)
As mentioned previously, gastrointestinal effects were identified as the most sensitive endpoint across assessments with a LOAEL of 175 mg/kg/day reported in EPA's draft human health risk assessment.

ERG's literature search identified six recent (2019-2013) *in vivo* studies on gastrointestinal toxicity published since the agencies completed their assessments, including three studies on glyphosate technical. Two of these studies found changes in the microbiome of rats following exposure to glyphosate technical, though it is unclear if agencies that issue major assessments would consider these adverse effects (Mesnage et al. 2021c; 2022a). A third study on piglets did not observe changes in intestinal morphology at doses up to 40 mg/kg/day (Qiu et al. 2020).

Considering the evidence presented in the recent literature, it is unlikely that the conclusions drawn by various agency assessments regarding glyphosate's gastrointestinal toxicity would be altered. Further, the recent literature identified very few articles describing associations between glyphosate technical and other effects (respiratory, cardiovascular, immunological, and hepatic).

2.6 Environmental Effects of Glyphosate

EPA's Office of Pesticide Programs (OPP) (EPA 2015a) and the European Commission (EC 2021) ecological assessments of glyphosate are the most recent and comprehensive environmental assessments available. This section first focuses on these assessments' conclusions and identified data gaps, because they informed the research that the ERG team conducted for this review. The two environmental assessments focus primarily on the fate and effects of technical glyphosate and its salts (i.e., the active herbicidal ingredient), although EPA considered certain GBFs as well.

EPA's ecological risk assessment considered glyphosate exposures that can occur from proposed labeled uses. The Agency considered available toxicity data (both for glyphosate technical and GBFs) to assess the range of risks from pesticide products that contain glyphosate, and that assessment considered toxicity of various formulation components, including surfactants (e.g., POEA). EPA and others determined that glyphosate, when used at maximum application rates for certain applications, is toxic to certain biota especially plants, including algae, bacteria, and fungi. However, EPA (2015a; 2021) and many recent peer-reviewed studies summarized in this scientific review (e.g., Mullin 2015; Martins-Gomes et al. 2022) reported that commercial formulations, such as Roundup, may be more toxic than the active ingredient to many species of plants and animals. In their evaluation of GBFs, EPA focused on evaluating POEA in aquatic habitats because the ingredient poses greater toxicity to aquatic animals than glyphosate; and EPA (2015a) noted that terrestrial uses allow for the application of formulations that contain surfactants such as polyethoxylated tallow amines or organosilicone surfactants (Mullin 2015). These substances were recognized as toxic to aquatic life, and therefore restricted in formulations designated for direct aquatic use. Recent research suggests, however, that formulation adjuvants, such as surfactants in commercial formulations containing glyphosate, may be toxic to certain terrestrial biota as well (e.g., Mullin 2015; Jacques et al. 2019; Ghandi et al. 2021; Straw et al. 2021).

In its *Biological Evaluation for Glyphosate*, EPA (2021) states: "Based on the comparison of technical and formulated glyphosate presented above, the toxicity observed in the formulation studies could be at least partially driven by the surfactants. However, other formulations may contain a different class of surfactant or no surfactant at all. The nature of the surfactant included in the formulation is considered Confidential Business Information (CBI) and is not included on product labels." This report summarizes how glyphosate technical (rather than GBFs) affected biota, to the extent information is available in assessments and the peer reviewed literature.

This review first summarizes the conclusions, information gaps, and uncertainties reported in the most recent EPA and EFSA assessments (Section 2.6.1). The review then describes the methodology used to search for evidence of environmental effects in the recent published literature (Section 2.6.2). Additionally, this section summarizes EPA's findings on the potential impacts of glyphosate on threatened and endangered species (Section 2.6.3). This section concludes with a concise summary statement on evidence for environmental effects of glyphosate (Section 2.6.4).

Throughout this section, references to "glyphosate" pertain to glyphosate technical, not to GBFs.

2.6.1 Review of Scientific Assessments

The following sub-sections summarize findings of major assessments conducted by EPA and in the European Union. This section concludes by commenting on data gaps in the assessments, which were used to inform the literature search discussed in Section 2.6.2.

2.6.1.1 EPA Registration Review – Preliminary Exposure Risk Assessment for Glyphosate and Its Salts (2015)

In 2015, EPA published a preliminary ecological risk assessment as part of the Registration Review process of glyphosate. The risk assessment evaluated the potential risks of glyphosate and its salts to non-target organisms based on the maximum application rate and the minimum application interval according to the label. It incorporated available exposure and effects data up through 2014, and the most current modeling and risk methodologies were conducted.

EPA (2015a) evaluated the potential risk to aquatic non-target organisms by estimating exposures from glyphosate only (spray drift, spray drift and runoff, direct application to water), glyphosate formulations (spray drift, direct applications to water), and the surfactant, POEA (spray drift). Additionally, EPA (2015a) evaluated the potential risk to terrestrial non-target organisms from glyphosate only (spray drift, direct contact) and glyphosate formulations (spray drift, direct contact). EPA (2015a) also evaluated multiple application rates with respect to terrestrial uses of glyphosate, including 3.75-, 8-, and 40-lb. acid equivalents (a.e.) per acre (A) with a different number of applications and different application intervals. This evaluation also estimated glyphosate concentrations in surface waters due to spray drift at a zero-foot buffer and with a 500-foot buffer, considering a range of application rates.

In characterizing the fate of glyphosate, EPA (2015a) noted that the major route of environmental transformation is microbial degradation, and the major product of glyphosate degradation is AMPA. Based on available toxicity data, EPA (2015a) concluded that AMPA, although a major glyphosate degradation product, is not considered a residue of toxicological concern in aquatic environments. Some recent research (e.g., Van Bruggen et al. 2018; Iori et al. 2020), however, indicates that there is uncertainty in terms of long-term effects of AMPA on aquatic organisms and other biota, as Section 2.6.3 describes further.

In the preliminary risk assessment, EPA (2015a) concluded that there were "No Impacts Anticipated" of glyphosate technical on the survival, growth, or reproduction of:

- Aquatic invertebrates, fish, aquatic non-vascular plants, or submerged vascular plants exposed to glyphosate in surface waters resulting from spray drift from adjacent fields (Table 3).
- Aquatic invertebrates or fish in surface waters where glyphosate was directly applied (Table 3).

TABLE 3. EPA RISK ASSESSMENT FINDINGS FOR AQUATIC ORGANISMS (EPA 2015a)

Taxa Group	Glyphosate Application Scenario	
	Spray Drift (Residue in water)	Direct Application
Aquatic Invertebrates	No Impacts Anticipated*	No Impacts Anticipated
Aquatic phase amphibians	No Impacts Anticipated	No Impacts Anticipated
Fish	No Impacts Anticipated	No Impacts Anticipated
Aquatic non-vascular plants	No Impacts Anticipated	May impact**
Submerged vascular plants	No Impacts Anticipated	May impact
Aquatic emergent vascular plants	May Impact	May Impact
Aquatic emergent non-vascular plants	May Impact	May Impact

* "No impacts anticipated" is defined as a Risk Quotient (RQ) < 1.0 based on modeling of glyphosate surface water concentrations and the most sensitive chronic toxicity threshold reported for the taxa group.

** "May impact" is defined as an RQ >1.0 based on modeling of glyphosate surface water concentrations and the most sensitive chronic toxicity threshold reported for the taxa group.

In addition, EPA (2015a) concluded that based on endocrine screening tests, the weight of evidence evaluation demonstrated no convincing evidence of the potential interaction of glyphosate with the estrogen, androgen, or

thyroid pathways in terrestrial mammals or terrestrial non-mammalian wildlife; EFSA (2017) reached the same conclusion. Some more recent studies, however, reported the possibility of potential endocrine effects of glyphosate at realistic environmental concentrations, as discussed later in this review (e.g., Mesnage et al. 2017; Smith et al. 2019).

EPA (2015a) also concluded that glyphosate "May Impact" survival and sub-lethal endpoints (biomass, growth, and reproduction) in exposure to:

- Aquatic emergent vascular plants (spray drift and direct application to aquatic environments) and aquatic emergent non-vascular plants (direct application to aquatic environments only) (Table 3).
- Birds, due to residues directly deposited or from spray drift on foliage resulting from direct deposition or spray drift, which may impact growth but not reproductive parameters (Table 4). (Note: EPA uses birds as surrogates for terrestrial-phase amphibians.)
- Terrestrial mammal growth and reproduction, due to glyphosate residues on foliage resulting from direct deposition or spray drift from aerial application to sugar cane as well as for most ground application uses up to the combined maximum annual rate (Table 4). (These results do not apply to humans because the highest body weight modeled was 1,000 grams and because they are based on food sources not representative of a human diet.)
- Upland plant and riparian/wetland plant biomass and growth in areas adjacent to a treated field, due to exposure to glyphosate residues on foliage resulting from spray drift (Table 4).

TABLE 4. EPA RISK ASSESSMENT FINDINGS FOR TERRESTRIAL ORGANISMS (EPA 2015)

Taxa Group	Glyphosate Application Scenario	
	Direct Contact	Direct Deposition or Spray Drift on Foliage
Bird Growth	No Impacts Anticipated	May Impact
Bird Reproduction	No Impacts Anticipated	No Impacts Anticipated
Mammals Growth	No Impacts Anticipated	May Impact
Mammals Reproduction	No Impacts Anticipated	May Impact
Upland Plants	May Impact	May Impact
Riparian/Wetland Plants	May Impact	May Impact

EPA (2015a) identified several data gaps in the preliminary risk assessment with respect to multiple taxa groups. Data gaps included:

- The lack of appropriate toxicity data for certain biota.
- Much of the available toxicity data for some types of fauna were based on acute toxicity effects (lethality), with fewer data available regarding more sensitive sublethal effects, such as effects on reproduction or growth.
- High variability in toxicity test data for certain species and effects.

Uncertainties in the 2015 environmental risk assessment include:

- Uncertainty in the available terrestrial invertebrate data regarding potential risks to non-adult life stages for terrestrial invertebrates at currently registered application rates.
- Uncertainty regarding effects on survival, growth, and reproduction of honeybee larvae from exposure to glyphosate residues on foliage from direct deposition or spray drift at application rates ≥1.92 lb a.e.
- Uncertainty in the potential chronic effects of glyphosate on birds due to mixed study results and sparse available data.
- Uncertainty regarding sublethal effects of glyphosate on estuarine-marine organisms due to sparse available toxicity data.

In addition to direct toxicity effects of glyphosate on biota, EPA (2015a) discussed potential indirect effects, particularly regarding effects on monarch butterflies (*Danaus plexippus* L.). EPA (2015a; 2021) acknowledged that many publications highlight the importance of the common milkweed (*Asclepias syriaca L.*) as a critical food resource for monarch butterfly larvae; the publications also emphasize conservation of milkweed to preserve monarch butterfly populations. The publications noted that milkweed, a target weed on glyphosate product labels, may be exposed to glyphosate from direct applications, spray drift, and runoff. This is of concern due to glyphosate's non-selective herbicidal action, which results in plant death of many species, including milkweed (EPA 2015a; 2021).

On its web site, EPA discusses its approach for protecting monarch butterflies; and in its Risk Management Approach to Identifying Options for Protecting the Monarch Butterfly, EPA addresses other issues surrounding monarch butterflies, including their observed population decline in the U.S. and the recent categorization of the monarch butterfly as a candidate for listing as a threatened and endangered species (https://www.fws.gov/media/monarch-butterfly-species-status-assessment-ssa-report).

Based on public comments received, to address monarch butterfly protection and conservation efforts, EPA is currently considering modifications to pesticide label language to increase awareness of monarch butterflies and their dependence on milkweed for their survival and reproduction. Specific examples of modifications under consideration include cooperative efforts between EPA and federal, state, and other stakeholders, education and outreach promoting spray drift management, best management practices, and integrated pest management. While this report focuses on recent relevant, peer-reviewed literature regarding indirect glyphosate effects on monarch butterflies, this report also mentions direct effects on other important pollinators, such as honeybees, bumble bees and other types of native bee species (see also Section 2.6.2.5).

2.6.1.2 EPA Biological Evaluation (2021)

EPA's 2021 Biological Evaluation (BE) was produced in response to the Ninth Circuit Court of Appeals decision, which ruled in favor of stakeholder's comments that EPA's preliminary risk assessment (EPA 2015a) did not evaluate the effects of glyphosate on federally listed threatened and endangered species. To address this issue, the 2021 BE assessed glyphosate risk to listed species by using known locations and critical habitats of federally listed species (aquatic and terrestrial listed plants and animals) and modeling glyphosate exposure concentrations in those habitats or locations. In contrast, the preliminary risk assessment primarily relied on laboratory toxicity data and modeled exposure scenarios for assessing risks to listed species; and the preliminary risk assessment was not geographically based.

It should be noted that EPA and its federal partners have put forth various efforts over recent decades to ensure the pesticide registration process meets Endangered Species Act requirements. Those efforts resulted in EPA recently issuing a workplan documenting how protecting wildlife (including threatened and endangered species) will be balanced with responsible pesticide use (EPA 2022a). While this workplan was issued after the BE was published, the Interim Decision for Glyphosate (EPA 2020a) indicates that EPA had already been working with its federal partners and other stakeholders to implement interim approaches for assessing risks to listed species and their designated critical habitats. Appendix D of the Interim Decision provides further details on the interim approaches that were applied.

In the 2021 BE, EPA concludes that technical glyphosate is "practically acutely non-toxic to terrestrial and aquatic animals" (including mammals, birds, amphibians, reptiles, fish, plants, and aquatic invertebrates) based on maximum allowable application rates for all uses (EPA 2021). This finding was based on a review of all registered glyphosate uses and all approved labels for herbicide products containing glyphosate. In a review of acute toxicity studies comparing the effects of technical glyphosate and GBFs, EPA (2021) reported that some GBFs were less toxic than glyphosate alone while other formulations were up to two orders of magnitude more toxic. EPA found GBFs to be:

- Moderately to highly acutely toxic to fish.
- Highly to very highly acutely toxic to aquatic invertebrates.
- Moderately acutely toxic to mammals.
- Slightly acutely toxic to birds.

In chronic toxicity studies of glyphosate technical and GBFs, EPA (2021) reported that various growth and reproductive effects in both terrestrial and aquatic animals have been observed. EPA also noted that direct application of glyphosate demonstrates adverse effects on growth to both vascular and non-vascular aquatic plants as well as terrestrial plants, as expected, due to its mode of herbicidal action.

EPA's 2021 BE made glyphosate effects determinations (NE, MA, NLAA, or LAA)[1] for 1,795 listed threatened or endangered species and 792 designated critical habitats. The first two steps in this process are designed to assess whether any "individual of a listed species is reasonably expected to be exposed to a pesticide at a level that results in a discernable effect" to an individual organism; and this approach is also applied to designated critical habitats. During these first two steps, EPA invokes a range of protective assumptions to ensure that risks are not underestimated.

- EPA reports that Step 1 "uses conservative assumptions and is intended to screen out species that are not reasonably expected to be exposed to the pesticide because they are outside of the pesticide use area, or when no environmentally discernable effect is expected to occur" (EPA 2021). In Step 1, EPA evaluated whether the registered uses of glyphosate will have No Effect (NE) or whether the uses May Affect (MA) an individual species or habitat. No NE determinations were made in EPA's BE for any species (Table 5) or designated critical habitats (Table 6). Species or habitats that are screened out in Step 1 are not considered further.
- In Step 2, EPA used a more refined approach to evaluate the MAs from Step 1 to determine if (a) glyphosate may affect but is Not Likely to Adversely Affect (NLAA) or (b) may affect and is Likely to Adversely Affect (LAA) a given species or habitat. This approach considers a range of additional information, including "life history information, actual pesticide applications (usage data), additional toxicity data, and a range of potential exposure concentrations" (EPA 2021). EPA made NLAA determinations for 119 species and 33 critical habitats and LAA determinations for 1,676 species and 759 critical habitats (Table 5 and Table 6). It should be noted that the LAA determinations in Step 2 are based on highly conservative analyses, and these determinations do not mean that entire species are in jeopardy or that critical habitats are being adversely modified. Rather, the LAA determinations are primarily intended to identify the subset of issues to be further investigated in Step 3. In that step, which is currently under way for glyphosate, EPA consults with other agencies (e.g., National Marine Fisheries Service, National Forest Service) for a Biological Opinion on whether potential effects to individuals might negatively impact populations or the entire species or adversely impact a critical impact and on whether risk mitigation measures are warranted.

In Step 2, EPA used a weight of evidence analysis to bin each LAA determination into one of three categories: strongest, moderate, and weakest relationship between glyphosate exposure and biological effects (Table 7). Of the LAA determinations, strongest evidence was found for only one species (the California clapper rail) and for six critical habitats. These amount to less than 1 percent of the species and habitats that were evaluated. The habitats with strongest evidence for effects included one for birds and five for plants (see footnote of Table 7 for details). However, none of the species and critical habitats with the strongest evidence for glyphosate-related effects are present or listed in Massachusetts.

On the other hand, the weakest evidence was found for 4 percent of species and 3 percent of critical habitat LAA determinations. The majority of LAA determinations (96 percent of species and 97 percent of critical habitats) were considered to have moderate evidence, with non-agricultural uses being the main risk drivers. Examples of the non-agricultural uses include applying glyphosate to non-cultivated land, open space developed land, rights of way, developed land, and for forest tree management; it also included consumer applications and residential uses. EPA acknowledged uncertainties associated with reliable usage data for the range of non-agricultural applications (EPA 2021).

[1] NE = No effect. MA = May affect. NLAA = Not likely to adversely affect. LAA = Likely to adversely affect.

TABLE 5. SUMMARY OF SPECIES EFFECTS DETERMINATIONS OF GLYPHOSATE (COUNTS BY TAXON) (EPA 2021)

Taxon	Step 1 Effects Determinations		Step 2 Effects Determinations		Totals
	No Effect	May Affect	Not Likely to Adversely Affect	Likely to Adversely Affect	
Mammals	0	99	24	75	99
Birds	0	108	20	88	108
Amphibians	0	36	0	36	36
Reptiles	0	47	14	33	47
Fish	0	190	11	179	190
Plants	0	948	8	940	948
Aquatic Invertebrates	0	207	22	185	207
Terrestrial Invertebrates	0	160	20	140	160
Total	0	1,795	119	1,676	1,795
Percent of Total	0%	100%	7%	93%	

TABLE 6. SUMMARY OF CRITICAL HABITAT EFFECTS DETERMINATIONS BY GLYPHOSATE (COUNTS BY TAXON) (EPA 2021)

Taxon	Step 1 Effects Determinations		Step 2 Effects Determinations		Totals
	No Effect	May Affect	Not Likely to Adversely Affect	Likely to Adversely Affect	
Mammals	0	33	6	27	33
Birds	0	31	1	30	31
Amphibians	0	25	0	25	25
Reptiles	0	16	6	10	16
Fish	0	107	2	105	107
Plants	0	460	4	456	460
Aquatic Invertebrates	0	71	3	68	71
Terrestrial Invertebrates	0	49	11	38	49
Total	0	792	33	759	792
Percent of Total	0%	100%	4%	96%	

TABLE 7. CLASSIFICATION OF LAA DETERMINATIONS BY STRENGTH OF EVIDENCE (EPA 2021)

Strength of LAA Call	Species Range		Critical Habitat	
	Number	% of LAA Determinations	Number	% of LAA Determinations
Strongest Evidence of LAA	1[a]	<1%	6[b]	<1%
Moderate Evidence of LAA	1,605	96%	733	97%
Weakest Evidence of LAA	70	4%	20	3%

[a] – California clapper rail (*Rallus longirotris obsoletus*)

[b] – Critical habitats for Mississippi sandhill crane (*Grus canadensis pulla*); Hoover's spurge (*Chamaesyce hooveri*), Gypsum wild-buckwheat (*Eriogonum gypsophilum*), Greene's tuctoria (*Tuctoria greenei*), Willamette daisy (*Erigeron decumbens*); and Large-flowered woolly meadowfoam (*Limnanthes pumilla* ssp. *Grandiflora*).

2.6.1.3 European Union Glyphosate Environmental Assessments

In the EU, EFSA and ECHA are responsible for reviewing and assessing data regarding glyphosate effects and determining whether glyphosate should be approved for certain uses. Their assessments were published by the European Commission (EC) (EC 2021). Glyphosate approval for use had been extended to December 2023, when peer review comments on the latest risk assessments were to be completed. On November 29, 2023, the EC authorized the use of glyphosate through 2033.

Several materials developed in the EU were reviewed for this section, including the EC *Combined Draft Renewal Assessment Report* originally published in 2009 and updated in 2021 (EC 2021), which was based on EFSA's risk assessment and ECHA's hazard assessments of glyphosate. The ERG team also reviewed EFSA's assessment of glyphosate effects in feed for animals (EFSA 2018b). Additionally, in 2019, four Member States (France, Hungary, the Netherlands, and Sweden) were appointed to act jointly as rapporteurs to evaluate the next application for renewal of approval; that evaluation is ongoing. The four Member States form the Assessment Group on Glyphosate (AGG). AGG reviewed the previous assessments and made recommendations regarding data gaps and the strength of previous conclusions (AGG 2021).

The following list briefly summarizes salient points in the 2021 draft EC *Combined Draft Renewal Assessment Report*. The final EC report was completed on November 29, 2023.

- *Birds.* EC (2021) concluded that the acute risk to birds was low for all use scenarios (e.g., agricultural, non-agricultural, rights of way, orchards, control of invasive species) based on the screening step; this finding is similar to EPA's conclusion (EPA 2015a). The EFSA 2018 report determined that additional consideration was needed for some scenarios in the long-term risk assessment. However, based on first-tier calculations, EC (2021) determined that the long-term risk to birds was low for all representative uses of glyphosate, and that higher-tier refinement was not needed for birds.
- *Mammals.* Consistent with EPA's conclusion (EPA 2015a), EC (2021) concluded that the acute risk of glyphosate to mammals was low. However, EC (2021) determined that the long-term risk to mammals was uncertain, and that further information was needed for the mammalian invertivore risk assessment (based on a scenario using voles) and all representative uses (e.g., agricultural, non-agricultural, rights of way, orchards, control of invasive species). EFSA (2018b) concluded that all representative use scenarios pose a low long-term risk for mammals, except for certain applications on railway tracks and for invasive species control, where a risk was still identified for voles. EFSA (2018b) reported that, assuming these two exceptions are targeted to specific areas and on plants or stand of plants, the long-term risk to mammals could be considered low for these uses.
- *Amphibians and Reptiles.* EC (2021) did not present risk assessment findings for amphibians and reptiles due to lack of relevant data for all potential uses (e.g., agricultural, non-agricultural, rights of way, orchards, control of invasive species). EC indicated that further consideration is needed regarding possible risk to reptiles following direct overspray in the field. EFSA issued a separate evaluation of potential impacts of glyphosate on amphibians and reptiles (EFSA 2018a). That evaluation concluded that effects on amphibians cannot be ruled out, even from low glyphosate exposure levels; it also found that an aquatic risk assessment may not be sufficiently protective for amphibians.

- *Aquatic Organisms.* EC (2021) noted that endpoints used for aquatic organism risk assessment are temporary, because of identified data gaps and the need to obtain further information. For aquatic plants, EFSA reported a need to have results for emergent macrophytes with different exposure designs, including overspray for potential uses (e.g., agricultural, non-agricultural, rights of way, orchards, control of invasive species). In addition, because glyphosate is persistent in sediment, EFSA concluded that a toxicity test with a rooted macrophyte is necessary to finalize the risk assessment for aquatic plants. A similar conclusion was determined by EFSA regarding effects of glyphosate and the degradation product AMPA on sediment dwelling organisms.

  Consistent with EPA's conclusions (2015), EC (2021) reported that aquatic algae are the most sensitive trophic level for glyphosate acute toxicity in aquatic organisms. For chronic toxicity, the lowest reliable chronic effect concentration was observed for a zebrafish species, *Brachydanio rerio*. AGG (2021) proposed that the risk assessment for aquatic organisms be revised to consider the updated concentrations for surface water and sediment and additional toxicity data. Based on the toxicity data considered at the time and the fact that glyphosate does not rapidly degrade, EFSA (2018b) and AGG (2021) classified glyphosate as Aquatic Chronic 2 and should be labelled as "toxic to aquatic life with long lasting effects."
- *Arthropods.* EC (2021) determined that direct exposure to glyphosate from all intended use patterns poses an acceptable risk to honeybees, because a margin of safety was demonstrated for chronic exposures to adult honeybees and to honeybee larvae. EC (2021) also noted that the hazard quotients (HQ) for acute contact toxicity for solitary bees were below the respective trigger value for different application rates. For arthropods other than bees, the EFSA risk assessment (EFSA 2017) also concluded an acceptable risk due to direct exposure, and this finding was based on multiple uses (e.g., agricultural, non-agricultural, rights of way, orchards, control of invasive species).
- *Soil Meso- and Macrofauna*. EC (2021) concluded that proposed glyphosate uses pose an acceptable risk to earthworms. This document also noted an acceptable risk for soil macro-organisms other than earthworms from the same proposed uses (e.g., agricultural, non-agricultural, rights of way, orchards, control of invasive species).

EFSA (2017) and the EU regulation 2017/2324 related to the approval of glyphosate stated: "Member States shall pay particular attention…to the risk to diversity and abundance of non-target terrestrial arthropods and vertebrates via trophic interactions." Regarding potential indirect effects, the EU notes that a loss of plant biodiversity following the application of plant protection products such as glyphosate may affect the presence of adequate habitats for arthropods, birds, and mammals. The loss of plant biodiversity might also affect survival of animals (arthropods, birds, mammals) that eat foliage; and it might affect nectar and pollen sources for bees.

EU notes that the current glyphosate risk assessments and protection goals address direct effects only and have not been defined to specifically address indirect effects. The EFSA (2017) document specifically notes data gaps regarding indirect effects to pollinators (e.g., bees), soil organisms, aquatic fauna, birds, and mammals. A specific example mentioned was potential indirect effects linked to the loss of habitats for arthropods and cascading effects to birds and mammals.

2.6.1.4 Summary of Data Gaps

The EPA and EU assessments, though extensive, have several data gaps that helped inform this project's literature search. Those data gaps include:

- Effects on soil microorganisms, and indirectly, rhizobiome productivity and diversity and soil health
- Indirect effects on the habitat of terrestrial invertebrates, particularly bees and monarch butterflies
- Chronic effects of glyphosate on amphibians, reptiles, and birds, for which available data were either sparse or ambiguous according to EPA sources (EPA 2015a; 2021)
- Potential anti-microbial resistance effects due to glyphosate
- Potential endocrine disruption effects of glyphosate
- Effects on Threatened and Endangered species

The following sections summarize information from the literature review on these topics.

2.6.2 Literature Review

This section summarizes the literature review that the ERG team conducted to compile findings from relevant peer-reviewed publications issued since the recent major assessments were completed. As noted above, the literature search focused on providing further context on the issues identified as potential data gaps in the assessments. This section first describes the literature search strategy (Section 2.6.2.1) and then reviews findings for different categories of organisms and endpoints (Sections 2.6.2.2 through 2.6.2.11).

2.6.2.1 Literature Search Strategy and Information Review Methodology

A keyword search of peer-reviewed journals and gray literature was completed in order to find the most up to date glyphosate toxicity information that addressed at least one of the data gaps above. Reports and peer-reviewed journals were limited by the following criteria:

- Lab and field studies that examined the effects of glyphosate as the active ingredient were reviewed. GBFs, such as Roundup or mixtures with other herbicides, were given low priority for review.
- Apical endpoints such as effects on growth, survival, reproduction, and development were preferred, as well as effects on behavior such as habitat avoidance. *In vitro* or biochemical effects were given lower priority unless they also examined an apical effect that could represent a population-level effect.
- While effects on all biota were considered, studies dealing with human health surrogates and endpoints were not included (because these studies are reviewed in the section on human health impacts).
- Studies published since EPA's 2015 draft risk assessment were primarily the focus of this review.

The Elsevier/ScienceDirect database was used to search for specific keywords. All combinations of exposure routes were organized as Boolean strings separated by "AND" to search the range of endpoints and responses. The full set of search terms and results is provided in Table 8.

Search results were focused primarily on published peer-reviewed journal articles and reports published between 2015-2023; for a few topics, older publications were reviewed due to a paucity of recent information. Results were limited to publications written (or already translated into) English. Citations and abstracts from the search were loaded to an EndNote Online Library, and duplicate citations were removed.

Inclusion criteria were first applied to the titles and abstracts, using best professional judgment. Resources that did not meet all inclusion criteria were excluded from further consideration. Full text PDF files of all articles that were deemed relevant in the initial screen were obtained through available library resources.

TABLE 8. TERMS USED FOR DATABASE SEARCHES

Keywords					Number of Elsevier/Science Direct Reviews since 2015	Number of Elsevier/ScienceDirect Reviews since 2015 (After Removing Duplicates)
Glyphosate	Toxicity	Birds	Direct		111	111
Glyphosate	Risk	Birds	Direct		110	15
Glyphosate	Toxicity	Birds	Indirect	Habitat	52	7
Glyphosate	Toxicity	Birds	Indirect	Food	119	10
Glyphosate	Toxicity	Amphibians	Direct		62	31
Glyphosate	Risk	Amphibians	Direct		64	3
Glyphosate	Toxicity	Amphibians	Indirect	Habitat	33	3
Glyphosate	Toxicity	Amphibians	Indirect	Food	69	2
Glyphosate	Toxicity	Reptiles	Direct		27	0
Glyphosate	Risk	Reptiles	Direct		27	0
Glyphosate	Toxicity	Reptiles	Indirect	Habitat	12	0

Keywords					Number of Elsevier/Science Direct Reviews since 2015	Number of Elsevier/ScienceDirect Reviews since 2015 (After Removing Duplicates)
Glyphosate	Toxicity	Reptiles	Indirect	Food	27	0
Glyphosate	Toxicity	Insects	Direct		339	235
Glyphosate	Risk	Insects	Direct		329	45
Glyphosate	Toxicity	Insects	Indirect	Habitat	100	0
Glyphosate	Toxicity	Insects	Indirect	Food	315	0
Glyphosate	Toxicity	Mammals	Direct		183	49
Glyphosate	Risk	Mammals	Direct		169	4
Glyphosate	Toxicity	Mammals	Indirect	Habitat	57	0
Glyphosate	Toxicity	Mammals	Indirect	Food	164	0
Glyphosate	Toxicity	Aquatic Inverts	Direct		131	131
Glyphosate	Risk	Aquatic Inverts	Direct		129	6
Glyphosate	Toxicity	Aquatic Inverts	Indirect	Habitat	69	0
Glyphosate	Toxicity	Aquatic Inverts	Indirect	Food	123	0
Glyphosate	Toxicity	Terrestrial Inverts	Direct		80	0
Glyphosate	Risk	Terrestrial Inverts	Direct		80	0
Glyphosate	Toxicity	Terrestrial Inverts	Indirect	Habitat	53	4
Glyphosate	Toxicity	Terrestrial Inverts	Indirect	Food	76	2
Glyphosate	Toxicity	Plants	Direct		744	342
Glyphosate	Risk	Plants	Direct		698	58
				Total	**4,552**	**1,058**

The following sections summarize the relevant 2015-2023 publications that were retrieved from the literature search. The summaries are organized by different categories of organisms (e.g., soil microbes, aquatic invertebrates, etc.).

2.6.2.2 Soil Microbe Effects

Glyphosate inhibits the 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS) enzyme on the shikimate pathway that is essential for the biosynthesis of some aromatic amino acids in bacteria and fungi, as well as in plants (EPA 2021). However, not all soil microbes are sensitive to glyphosate, depending on the class of EPSPS they produce (van Bruggen et al. 2018). EPA (2015a) discussed the fate of glyphosate and its major degradation product, AMPA, in soil and water to derive predicted exposure concentrations that were used in both the preliminary risk assessment (EPA 2015a) and Biological Evaluation (EPA 2021). Glyphosate is ionic and known to chelate with various metal ions; and AMPA is ionic and can form metal complexes with Ca^{2+}, Mg^{2+}, Mn^{2+}, Cu^{2+}, and Zn^{2+} (EPA 2015a). Batch equilibrium data for AMPA indicate high sorption to soils. Laboratory and field dissipation data indicate that AMPA is substantially more persistent than glyphosate and that both glyphosate and AMPA sorb strongly to soil (EPA 2015a), leading some researchers to postulate detrimental effects on soil microbes.

In their review of glyphosate degradation processes, Martins-Gomes et al. (2022) noted that many soil properties may affect degradation rates and persistence of glyphosate in soil. In its glyphosate ecological risk assessment, EFSA reported that glyphosate's DT50 (a measurement of the time necessary to degrade half of its original concentration) is highly variable depending on the soil characteristics. For example, under anaerobic conditions, glyphosate DT50 varies from 135 to >1000 days, indicating high persistence, while under aerobic conditions, laboratory studies reported DT50 values ranging from 1.01 to 67.72 days (EFSA 2015). EPA (2015a) also reported slower degradation rates and higher persistence of glyphosate under anaerobic soil conditions, suggesting that in certain types of soils (e.g., many clay soils, some wetland sediments, and other soil types that tend to be more anaerobic), glyphosate may persist—and the microbial community may be exposed for longer periods of time.

Due to the widespread use of glyphosate products and potential persistence in soils under certain conditions, several researchers (e.g., Gaupp-Berghausen et al. 2015; Helander et al. 2018; Zhan et al. 2018; van Bruggen et al. 2021; Ruuskanen et al. 2023) and some conservation groups (e.g., Soil Association Scotland [not dated]) have raised concerns regarding potential effects of glyphosate on soil microbial populations, and thereby potential indirect effects on plants and possibly other biota.

Reviews of peer-reviewed published studies by van Bruggen et al. (2018; 2021) noted much uncertainty as to whether glyphosate, applied at labeled application rates, results in deleterious effects on microbial communities and their activities in the soil and rhizosphere. For example, van Bruggen et al. (2018) reported that in some studies (reviewed also by Gill et al. 2018), glyphosate treatments (applied at recommended or lower dosages) negatively affected microorganisms that promote plant growth, such as *Burkholderia* spp., *Pseudomonas* spp., arbuscular mycorrhizal fungi, and nitrogen fixing *Rhizobium* spp. These treatments resulted in reduced plant growth or changes in grassland vegetation cover and composition. Singh et al. (2020) noted in their review that glyphosate applications altered the soil texture and microbial diversity by reducing the microbial richness and increasing the population of phytopathogenic fungi. De Maria et al. (2006) reported that glyphosate may disrupt host plant growth and survival via indirectly disturbing nitrogen metabolism or directly harming the symbiotic rhizobial bacteria present in the soil.

In other studies, comparing soil treated with glyphosate active ingredient and untreated control soil, microbial communities appeared to recover from short-term glyphosate treatment with only minor or no effect on microbial structure, biomass, or activity (e.g., Haney et al. 2000). Further, other researchers investigated glyphosate's impact on soil microbial communities, and their field-based study found that "plots treated with glyphosate did not differ from untreated plots in overall microbial community composition after controlling for other factors" (Kepler et al. 2020); and a more recent review found that glyphosate's impacts to soil microbial processes are "very small" and that most studies that evaluated how glyphosate and GBFs affect soil microbial community structure and function found no effects (Rodríguez-Gil et al. 2021). EPA (2015a) reported that some studies have shown that glyphosate can increase microbial respiration in the short term (seven to 38 days), and other studies reported no effect of glyphosate on microbial respiration. EPA (2015a) also noted that soil organic matter may indirectly affect the sorption of glyphosate to soil. Phosphate present in soil competes with glyphosate for sorption, which ultimately affects the retention and degradation of glyphosate. Singh et al. (2020) and Busse et al. (2001) report that many species of bacteria and fungi in soils may use glyphosate as a phosphorus source especially at higher glyphosate concentrations, thereby degrading glyphosate and furthering microbial growth. Busse et al. (2001) reported that while glyphosate was toxic to bacteria and fungi in soil-free medium, when added to soil at environmentally realistic concentrations, glyphosate had no measurable effect on soil microbe respiration or vegetation productivity.

In summary, there is mixed evidence regarding effects of glyphosate on soil microbial community productivity and thereby plant health. Some studies report that the soil microbial community may be affected under certain soil conditions and for short periods of time (days). However, in its response to public comments on the preliminary ecological risk assessment, EPA also noted "other reported information suggested that adverse impacts to soil organisms is anticipated to be low" (EPA 2018b). The degree to which glyphosate affects long-term soil condition and plant productivity is uncertain based on current published studies, and many of the publications reviewed for this section note that this is an area in need of further research.

2.6.2.3 Aquatic Invertebrates

Glyphosate's mechanism of herbicidal action involves inhibiting protein synthesis by blocking the shikimate pathway—a pathway that is absent in animals. As a result, glyphosate is practically nontoxic to aquatic invertebrates in acute exposure tests (EPA 2015a). Potential indirect glyphosate-related toxic effects (e.g., due to deleterious changes in habitats or vegetation used for food) are less well understood. The literature search did not identify peer-reviewed publications (2015-2023) that met the search criteria and addressed glyphosate effects on aquatic invertebrates.

Most of the recent publications that addressed aquatic invertebrates tested GBFs and not glyphosate itself. Linz et al. (1999), for example, studied potential indirect effects of a GBF on wetland invertebrates due to glyphosate-induced reduction in cattail coverage. Their results suggested that certain populations of aquatic invertebrates

appeared to be enhanced by a reduction in cattail coverage with glyphosate-based herbicide, while other species declined. Specifically, Corixidae and Chironomidae were more abundant in the treated wetlands, which the authors suggested may have been in response to increased food availability due to decaying emergent vegetation and increased algal production caused by more sunlight availability (Linz et al. 1999). Chaoboridae, however, were consistently more plentiful in the reference (untreated) wetlands (Linz et al. 1999). The authors concluded that it is unlikely that glyphosate was exhibiting direct toxic effects to this species based on laboratory toxicity studies and field studies. Based on the available toxicity data, Linz et al. (1999) concluded that changes in vegetation composition or structure due to glyphosate-herbicide treatment caused indirect effects on chaoborids during certain phases of their life cycle; however, because the Linz et al. (1999) study examined a GBF rather than glyphosate itself, their results may have been due to co-formulants in the product tested.

2.6.2.4 Soil Invertebrates

Gao et al. (2021) showed that survival, development, pupation rate, and emergence rate of the soil beetle *Harmonia axyridis* was not significantly affected by low and high concentrations of glyphosate relative to recommended application rates, but glyphosate did significantly reduce the body weight of *H. axyridis*. They also found that glyphosate altered the bacterial endosymbiont community of *H. axyridis* by affecting the abundance of dominant soil bacteria (see also the following subsection on bees). Gao et al. (2021) further noted that the balance of symbiotic soil bacteria is critical to maintain the growth and development of insects. These authors reported that several studies demonstrated that glyphosate changes the gut microbiota of organisms because of its antimicrobial activity.

Stecca et al. (2016) found that glyphosate-containing herbicides had low toxicity in the parasitoid wasp *Telenomus remus*, inducing minor negative effects to growth, development, and reproduction. They concluded that neither the active ingredient glyphosate or co-formulants affected pupal or adult life stages of this species.

Based on recommended glyphosate concentrations for agricultural uses in Flanders (Belgium) and glyphosate concentrations reported in surface waters, Janssens and Stoks (2017) found that a seven-day exposure of 2 mg/L glyphosate had adverse effects on the survival rate, behavior, fat content, and other physiological indices of damselfly larvae. The authors noted that Roundup exposure resulted in greater toxicity effects, which were attributed to POEA and perhaps other co-formulants.

The life history parameters of the rose-grain aphid were significantly affected by a GBF (Saska et al. 2016). However, the study could not distinguish whether the effects observed were due to glyphosate or co-formulants in the herbicide tested.

Jacques et al. (2019) examined a comparative toxicology study that tested glyphosate and a GBF on the soil nematode, *Caenorhabditis elegans*. Their results showed that only the GBF caused significant negative effects on brood size, body length, oocyte size, and the number of apoptotic cells. They demonstrated that the addition of inert ingredients increased toxicity of glyphosate to *C. elegans*.

2.6.2.5 Pollinator Insects: Bees

Cullen et al. (2023) examined effects of glyphosate and a GBF on bumblebees (*Bombus terrestris*). Results of survival, behavioral, and consumption studies indicated no adverse effects of glyphosate; however, effects on digestive tract microbiota were observed using DNA sequencing of the digestive tract samples. Both glyphosate alone and co-formulants were found to influence cellular and physiological processes in the digestive tract, including alterations of proteins related to oxidative stress regulation, metabolism, cellular adhesion, and the extracellular matrix. Some differences were observed in the digestive tract microbiota and proteins in bees exposed to glyphosate versus the GBF. For instance, glyphosate alone affected proteins associated with endocytosis, oxidative phosphorylation, and carbohydrate, lipid, and amino acid metabolism. Glyphosate alone was also shown to alter structural, metabolic, and oxidative stress as well as mitochondrial proteins, whereas the GBF affected proteins associated with metabolism and the lysosome (Cullen et al. 2023).

Daisley et al. (2020) researched and described the ability of bee microbiota to withstand environmental stressors and their influence on host disease tolerance. Their research suggested that generational changes in microbiota after multiple exposures to glyphosate reduced the abundance of several symbiotic microbes in the gut of bees,

which may reduce bee immunity to diseases from viruses and other sources. Berenbaum and Liao (2019) determined that insecticides, herbicides (including glyphosate), and fungicides can concentrate in hives and become toxic to honeybees, but sublethal effects, if any, have been challenging to assess. Yoder et al. (2017) reported that fungal communities existing in bee microbiomes can be affected by fungicides intended for use on fungal plant pathogens.

Similar to the findings reported by Daisley et al. (2020), Motta et al. (2018) demonstrated that the microbiome of honeybees was affected by environmentally realistic concentrations of glyphosate during and after gut colonization due to effects of glyphosate on certain host bacteria. They further noted that glyphosate exposure during early gut colonization increased mortality of bees exposed to an opportunistic pathogen.

Straw et al. (2021) compared the effects of glyphosate alone, several GBFs, and non-glyphosate pesticide treatments to determine if glyphosate or co-formulants are responsible for bee mortality. Several lines of evidence revealed that co-formulants—and not glyphosate—were the cause of bee mortality. Motta et al. (2018) found that spraying honeybees with glyphosate did not cause mortality, reinforcing the findings of Straw et al. (2021). These findings suggest that mortality could potentially be reduced or avoided by substituting mixture alternatives with the active ingredient.

Though it is known to degrade rapidly in the environment, glyphosate may be accumulated in crops and soil (EPA 2015a). A literature review by Ledoux et al. (2020) investigated the effects of glyphosate and glyphosate co-formulant residues in honey on human health, bee health, and honey supply. The authors reported that residues of glyphosate co-formulants in honey could be more detrimental to animal (i.e., livestock, wildlife) health rather than residues of the active ingredient glyphosate. Berg et al. (2018) suggested four potential pathways through which glyphosate could impact the food supply for animals and humans. The pathways were direct application to agricultural food products, contamination via drift or water transport, disturbance during harvest and processing, and dissemination with animal movement. These authors point out that an example of the fourth pathway could occur when bees are exposed to glyphosate-treated weeds that attract pollinators, who then carry the glyphosate back to the hive where the honey is contaminated and possibly harvested by wildlife and humans (Karise et al. 2017).

Battisti et al. (2021) reviewed scientific reports between 1945-2020 and selected 34 datasets from a collection of sixteen scientific papers to conduct a meta-analysis on the toxicity effects of glyphosate on bees. Considerations investigated by the authors in their review included study methodology, whether exposure was via ingestion or contact, life stage of bees, and dosage compared to label uses prescribed by EPA. The authors noted mixed results from previous studies related to the toxicity of glyphosate on bees, ranging from little to no impacts to high mortality based on maximum glyphosate exposure in accordance with labeled uses. Their meta-analysis indicated differences in mortality between control groups and experimental groups of bees leading the authors to conclude that glyphosate caused increased mortality at maximum dosages prescribed by EPA.

Rodríguez-Gil et al. (2021) also reviewed risks of glyphosate and its principal metabolite to terrestrial invertebrates, including bees. This review identifies an important limitation for ecotoxicity tests involving GBFs: in the environment, glyphosate and various formulants degrade at different rates. Therefore, the profile of glyphosate and formulants in the environment likely differ from the profile found in the original GBFs used in the ecotoxicity tests. The authors concluded that "use of formulations of glyphosate under good agricultural practices presents a de minimis risk of direct and indirect adverse effects in non-target organisms," including terrestrial invertebrates.

Zioga et al. (2022) conducted a study examining effects of GBFs on the pollinator plants oilseed rape and blackberry (*Rubus fruticosus*), commonly found near edge habitat of agricultural fields in Ireland, and bee health. These authors noted that edge vegetation may be subjected to overspray from pesticide application, raising concern for potential transfer to the digestive systems of pollinators of the flowering plants. Though glyphosate as a treatment alone was not examined in this study, the authors recorded glyphosate residue in plant nectar and pollen from pollinating honeybees and bumblebees where a GBF was applied. Their study found glyphosate in blackberry samples taken 2-7 days after application, but no glyphosate was found two months after application; AMPA was not detected in any of these samples. Zioga et al. (2022) recommended further research into the

impacts of glyphosate on bees, specifically including a longitudinal study of the presence and fate of glyphosate on edge habitat vegetation several days following field application.

Tan et al. (2022) summarized recent developments regarding the relationship of glyphosate exposure to lethal or sub-lethal effects in honeybees. Their review described honeybee behavior, growth, development, metabolic processes, and immune defense in response to glyphosate exposure. While they noted that some researchers have reported effects on bee microbiome, the connection between glyphosate exposure and certain bee behaviors and metabolic processes were uncertain based on research to date. The authors described approaches for future investigation of the glyphosate-related toxicity to honeybees.

Weidenmüller et al. (2022) noted that honeybees use active thermogenesis to maintain a warm temperature in the hive, creating an environment conducive to reproduction and subsequent colony growth and survival. Their ability to engage in thermoregulation can be impacted when nectar-providing resources are scarce. Pollinator-attracting plants inhabiting edge-of-field borders may be affected by herbicide overspray or spray drift, resulting in a decline of nectar and pollen resources for honeybees. Weidenmüller et al. (2022) explored impacts of non-lethal (i.e., subacute) levels of glyphosate active ingredient on honeybee exposure to GBF applications. The authors reported that hive temperature conditions did not become problematic with individual declines in the ability to thermoregulate when resources were sufficient. However, when resources were in limited supply, beehives associated with GBF exposure experienced a 25 percent decrease in hive temperature when compared to hives without the herbicide exposure (Weidenmüller et al. 2022). It is not known whether the thermoregulation effect observed by these authors was due to the glyphosate active ingredient, co-formulants in the GBF examined, or would also occur with loss of floral resources from any herbicide use.

In summary, direct toxicity effects of glyphosate on bees appear unlikely based on the literature, glyphosate concentrations reported in the field, and glyphosate concentrations considered in EPA's ecological risk assessment based on various label applications. On the other hand, current research suggests a potential for indirect effects of glyphosate on bees due to direct effects on the bee microbiome, which protects individual immune response and perhaps other functions. The nature and extent of indirect effects of glyphosate on bees and other pollinator insects remains an uncertainty, as discussed further in the next sub-section.

2.6.2.6 Pollinator Insects: Butterflies

Butterflies (including *Danaus plexippus*, monarch butterflies) inhabit meadows, fields, roadsides, gardens, and other areas with flowering vegetation. Although the following discussion is specific to monarch butterflies, many other types of butterflies may have similar characteristics and also be potentially affected by glyphosate. Monarch butterflies are especially attracted to milkweed plants, which are prime habitat during all stages of the monarch life cycle. As adults, monarchs feed on the nectar of the milkweed and lay eggs on the leaves. When the eggs hatch, the caterpillars eat the leaves and gain a defense mechanism whereby they become toxic to most birds and mammals (USDA 2018).

In the past, the margins of agricultural fields or roads have provided preferrable growing conditions for milkweed, but the development of pesticide-tolerant crops has been reported to have deleterious effects on milkweed in those locations due to drift and overspray of herbicides, which do not affect the resistant crops, but do affect milkweeds and other non-resistant plants. A literature review by Prosser et al. (2016) cites a study conducted by Feber et al. (1996) concluding that spray drift and off-site transport of glyphosate herbicides to field edge habitats resulted in significantly fewer milkweed flowers and nectar sources. If good agricultural practices are being employed, edge-of-field habitats are more likely to be affected by spray drift than by direct application, with exposure severity varying vertically and horizontally with increased distance from the border (Prosser et al. 2016; EPA 2015a).

A study by Taylor et. al (2020) considered predictions of the "migration mortality hypothesis," which suggests that increased mortality during the fall monarch migration is responsible for the reduced populations of overwintering monarchs—and that the increased mortality is not due to decline in milkweed that occurs during the monarch summer breeding season. Using data from the Monarch Watch tagging program, Taylor et al. (2020) concluded the following: (1) the summer population and overwintering population sizes were correlated; (2) migration success was not significantly correlated with overwintering population counts; and (3) migration success has not decreased

over the last two decades. These conclusions are contrary to the migration mortality hypothesis predictions. Reported findings correlate migration success to greenness of milkweed nectar-harvesting locations in the southern U.S. and deduce that summer monarch populations should be positively impacted by increasing and protecting milkweed habitat (Taylor et al. 2020). Prosser et al. (2016) suggested that reduced populations of monarch butterflies in the Midwest were related to breeding ground loss owing to a decline in national populations of milkweed plants after the application of glyphosate herbicides on glyphosate-tolerant crops.

In summary, there is some evidence that glyphosate could have indirect effects on insect pollinators such as bees and butterflies due to (a) direct effects on the insect microbiome (e.g., in bees) which may affect disease resistance and (b) to loss of preferred vegetation sources for food or habitat (e.g., in butterflies, bees, and other insect pollinators). As noted in Section 2.6.1, EPA is implementing several activities involving research, modifications to glyphosate herbicide labelling in some cases, and outreach and communication efforts to further protect monarchs and other insect pollinators.

2.6.2.7 Sublethal Effects on Fish

Apart from the laboratory toxicity test data reviewed by EPA (2015a and 2021), relatively few additional studies that met the search criteria were identified regarding effects of glyphosate on early life stages of fish. A limited number of recent studies reported effects that indicate both genetic and behavioral changes in the early life stages. Smith et al. (2019) found that exposure to 0.5 mg/L glyphosate, either as Roundup or glyphosate alone, induced developmental, reproductive, and epigenetic effects in larval Japanese medaka. Glyphosate exposure increased developmental abnormalities in medaka fry and increased global DNA demethylation in the developing fry. Fecundity and fertilization efficiency, however, were not altered by exposure to glyphosate. Based on their results with Japanese medaka, Smith et al. (2019) suggested that fish could be at risk when exposed to glyphosate alone at environmentally relevant concentrations.

A study reported by Faria et al. (2021) indicated behavioral effects on zebrafish due to sublethal exposure to glyphosate (98 percent pure) at environmentally relevant concentrations. The authors reported significant impairment of exploratory and social behaviors in zebrafish consistent with increased anxiety after a two-week waterborne exposure of 0.3 and 3.0 µg/L glyphosate. Effects were also found within the brains of exposed fish indicating changes to the antioxidant defense system and increases in oxidative stress. These results suggest that concentrations of glyphosate found in aquatic systems may have detrimental effects on fish survival by decreasing exploration of the environment or alteration of social interaction. Faria et al. (2021) also found that only intergenerational glyphosate exposure significantly increased the susceptibility of rainbow trout to hematopoietic necrosis virus, suggesting that generational exposure to glyphosate induces developmental toxicity and increases viral susceptibility.

While the foregoing studies indicate potential physiological effects of glyphosate exposure on laboratory fish species, it is uncertain whether realistic glyphosate aquatic exposure regimes elicit the types of effects observed in laboratory studies. Specifically, the research has not characterized the exposure concentrations, durations, and frequencies for different glyphosate uses, including impacts from spray drift.

2.6.2.8 Sublethal Effects on Amphibians

Few recent studies were identified that met the search criteria for glyphosate effects on amphibians. In a review of various agrochemicals' ability to affect amphibians, Trudeau et al. (2020) identified multiple studies in which effects of GBFs were reported to have multiple endocrine effects (e.g., targeting both the thyroid and gonadal axes) in different amphibian life stages; however, their conclusions are based on exposure to GBFs and not glyphosate alone.

Bach et al. (2016) studied how exposure to glyphosate and the commercial formulation RoundUp ULTRA MAX® affected the South American Creole frog (*Leptodactylus latrans*) during Gosner stages 25 and 26 (tadpoles). They evaluated multiple endpoints, including mortality, swimming activity, growth, development, and the presence of morphologic abnormalities. They found that while the commercial formulation was much more toxic than glyphosate alone, adverse effects of glyphosate occurred on growth, development, and generation of some morphological abnormalities (e.g., oral irregularities and edema).

Wang et al. (2019c) exposed tadpoles (*Microhyla fissipes*) to the GBF "KISSUN®" (30 percent acid equivalent glyphosate) and measured mortality, morphological traits, and histopathological response. Sublethal exposure of GBF to *M. fissipes* resulted in a preference for higher water temperature, reduced growth, and modified tail position (positively correlated with locomotor activity) ratio, which the authors suggested could be used as ecotoxicological indicators for GBF contamination. However, it is unclear from this study whether the effects observed were due to glyphosate or co-formulants.

All papers cited in this section recommend further studies to determine whether glyphosate itself has detrimental sublethal effects on amphibians.

2.6.2.9 Sublethal Effects on Birds and Mammals

No relevant sources were identified in this review that met the search criteria and provided information regarding effects on birds and mammals that was not already reviewed by EPA (2015a) or by the EC. One study reported by Sorensen et al. (2021) found evidence of potential gut microbiota effects in livestock fed with feed containing glyphosate residues, but the authors indicate that longer-term *in vivo* studies are needed to truly understand the magnitude and extent of this effect.

In 2018, EFSA published a report assessing the health risks of farm animals in relation to the presence of glyphosate and its residues in feed. EFSA considered the impact of glyphosate residues in feed on bovine, ovine, equine, porcine, and avian species. The agency reported that glyphosate and its metabolite, AMPA, are not expected to have a negative impact on these species on the basis of available data (EFSA 2018a). Data from cattle and sheep exposures indicated no negative effect on ruminal microflora at a level that covers the maximum dietary burden for all authorized uses, except for, according to EFSA (2018a), the use of glyphosate on grass forage. Given the maximum dietary burden covering all uses of glyphosate, including grass foraging, EFSA concluded that glyphosate is not expected to have negative effects on microbial communities in the rumen that could affect the health of bovine and ovine species.

2.6.2.10 Antimicrobial Effects

As noted previously, certain types of bacteria and fungi are susceptible to glyphosate because they rely on the shikimate pathway to synthesize proteins (vanBruggen et al. 2018). Some researchers have suggested that glyphosate may have antimicrobial properties that could lead to effects on animals, although relevant peer-reviewed studies examining glyphosate itself (or its salts) are sparse. In their review of glyphosate effects on livestock feed, Sorensen et al. (2021) reported that glyphosate has antimicrobial and mineral-chelating properties such that dietary glyphosate residues may affect livestock gut microbiota and mineral status, potentially causing adverse effects on animal health and productivity. However, those findings are based on *in vitro* studies and *in vivo* studies using realistic glyphosate exposures are needed.

In their review of effects of glyphosate on biota, Meftaul et al. (2020) reported that glyphosate has been shown to induce antibiotic resistance in bacteria such as *Escherichia coli* and *Salmonella enterica serovar Typhimurium*, which may cause increased bacteria-based diseases in animals; however, these authors noted that resistance induction may be due to co-formulants such as POEA and not the active ingredient glyphosate. A similar conclusion was reported for bees by Daisley et al. (2020), in which the authors suggested that chronic exposure to antimicrobial xenobiotics can systematically deplete honeybees of their microbes and hamper cross-generational preservation of host-adapted symbionts that are crucial to health.

Gao et al. (2021) cited several peer-reviewed studies demonstrating that glyphosate's antimicrobial activity can change the gut microbiota of organisms, which may have deleterious effects on organisms such as bees. The symbiotic relationship between certain bacteria and aquatic organisms such as marine mussels was compromised due to glyphosate or AMPA exposure (Iori et al. 2020). These authors suggested that both the effects of direct toxicity and changes occurring in the host-microbial community need to be taken into consideration to determine the overall ecotoxicological hazard of glyphosate.

Ospino et al. (2023) conducted a study examining resistance to several antibiotics and found that glyphosate did not affect the minimum inhibitory concentration of the tested antibiotics, but glyphosate did enhance bacterial tolerance and persistence. They concluded that, by inducing aromatic amino acid starvation (via blocking the

shikimate pathway), glyphosate contributes to the temporary increase in *E. coli* tolerance or persistence but does not affect antibiotic resistance.

In summary, there have been reports of glyphosate's effects on microbial resistance to antibiotics and potential disruption of host-microbial symbiosis in some micro-organisms. However, researchers have recommended more in-depth studies examining whether glyphosate, its co-formulants, or both can lead to antibiotic resistance at recommended application rates for different uses.

2.6.2.11 Endocrine Disruption Effects

EPA and EFSA reported that glyphosate is not expected to cause endocrine disruption effects on biota based on screening test results. Other researchers, however, have said this issue is still under debate. For example, in their review of glyphosate effects on the soil biome, Ruuskanen et al. (2023) postulated that altered gut microbiomes reported in some research may directly affect pathogen resistance, endocrine disruption, and therefore, animal survival and reproduction. Smith et al. (2019) conducted physiological and genetic analyses of larval *Medaka* fish exposed to 0.5 mg/L glyphosate, either as 98 percent glyphosate or as Roundup. They reported that glyphosate induced developmental, reproductive, and epigenetic effects in larval fish, suggesting that fish could be at risk for endocrine disruption due to glyphosate exposure.

On the other hand, Defarge et al. (2018) noted in their study testing glyphosate and other GBF ingredients that the toxic effects and endocrine disrupting properties were mostly due to the formulants (e.g., surfactants such as POEA) and not to glyphosate alone. Tóth et al. (2020) reported similar results after examining direct hormonal activity (estrogenic and androgenic effects measured by *Saccharomyces cerevisiae* BLYES/BLYAS strains, respectively) of glyphosate, AMPA, POEA, and thirteen GBFs in which eleven formulations do not contain POEA. They found that toxicity and endocrine disruption effects were linked to co-formulants and not to glyphosate itself (Tóth et al. 2020). Finally, in their review of literature on herbicide toxicity to amphibians, Trudeau et al. (2020) noted that several agrichemicals, including GBFs, have multiple endocrine effects (e.g., targeting both the thyroid and gonadal axes); however, their conclusions are based on exposure to GBFs and not glyphosate alone.

In summary, while some recent reports comment on potential endocrine effects associated with glyphosate impacts to biota, it appears that at least some of these effects may be due to certain surfactants and other chemicals in GBFs. Several of these studies recommended that researchers test co-formulants used in commercial products to ensure protection of biota where GBFs (and other herbicides) are used.

2.6.3 Consideration of Threatened and Endangered Species in Massachusetts

According to MassWildlife's Natural Heritage and Endangered Species Program website, Massachusetts has a wide variety of plants and animal species, with some unique species that occur naturally in the state. Under the Massachusetts Endangered Species Act (MESA), 173 species of animals and 259 species of plants are currently listed as Endangered (E), Threatened (T), or of Special Concern (SC) (Table 9). Of these 432 species, 27 are also listed as federally endangered or threatened. (Note: The blue-spotted salamander and the Atlantic sturgeon are counted as one species. The blue spotted salamander is listed as Threatened in Bristol and Plymouth counties and as Special Concern in other counties. The Atlantic sturgeon is listed as Federally Endangered in the Connecticut River and as Federally Threatened in the Merrimack River.) These species are either at risk or may become at risk of extinction. Rarity in the state, population trend, and overall threat are the main criteria used to determine extinction risk.

TABLE 9. SUMMARY OF THE MESA LIST (AS LISTED IN 321 CMR 10.90, JANUARY 10, 2020), 432* NATIVE PLANT AND ANIMAL SPECIES ARE PROTECTED UNDER THE MASSACHUSETTS ENDANGERED SPECIES ACT (M.G.L. C. 131A).

Taxonomic Group	Endangered	Threatened	Special Concern	Totals
Mammals (including 6 whales)	11 (6 FE, 1 FT)	0	3	**14**
Birds (breeding)	9 (1 FE)	7 (2 FT)	14	**30**
Reptiles (including 5 sea turtles)	8 (4 FE, 1 FT)	5 (2 FT)	3	**16**
Amphibians	0	3	2	**4***
Fish	4 (2 FE)	2 (1 FT)	4	**10**
Invertebrates (non-marine only)	32 (2 FE, 2 FT)	25	42	**99**

Taxonomic Group	Endangered	Threatened	Special Concern	Totals
Plants (vascular)	153 (3 FE, 1 FT)	66	40	**259**
Totals	**217 (18 FE, 5 FT)**	**108 (5 FT)**	**108 (0)**	**432* (28 FE or FT**)**

*Blue-spotted Salamander (*Ambystoma laterale*) counted as one species. It is Threatened in Bristol and Plymouth Counties and Special Concern in other counties.

**Atlantic Sturgeon (*Acipenser oxyrinchus*) counted as one species. It is Federally Endangered in the Connecticut River and Federally Threatened in the Merrimack River.

FE = species listed under the U.S. Endangered Species Act as Federally Endangered.
FT = species listed under the U.S. Endangered Species Act as Federally Threatened.

The remainder of this section provides further context on the different taxonomic groups of T&E species and species of special concern with respect to glyphosate:

- *Mammals*. There are currently eleven endangered mammalian species listed in Massachusetts and an additional three listed as of special concern. Six of these are whales and are assumed to have little or no exposure risk to glyphosate. The other eight mammalian species may have limited exposure directly to glyphosate, but indirect effects on potential food resources (e.g., plants used for food) or habitat (e.g., plants used for shelter) may be relevant. EPA's BE (2021) concluded that, among the 99 federally listed mammals that were evaluated, 75 are likely to be adversely affected by glyphosate; EPA also concluded that 27 of the 33 critical habitats determined for mammals are likely adversely affected. As noted previously, these likely to adversely affect (LAA) determinations are based on highly conservative analyses, and they do not mean that entire species are in jeopardy or that critical habitats are being adversely modified. Rather, these determinations are primarily intended to identify the subset of issues to be further evaluated by EPA in consultation with other agencies (e.g., National Marine Fisheries Service, National Forest Service) for a Biological Opinion on whether potential effects to individuals might negatively impact populations or the entire species or adversely impact a critical impact.

  State-listed mammal species that typically occur in transportation rights of way or near agricultural fields include eastern small-footed bat, little brown bat, northern long-eared bat, Indiana bat, tricolored bat, rock shrew, water shrew, and southern bog lemming. These species could be potentially at risk from glyphosate exposure; however, the acute risk to mammals in general is likely to be low based on toxicity data reviewed by EPA, EC, and others.

- *Fish*. There are currently ten species of fish listed as endangered (n=4), threatened (n=2), or of special concern (n = 4) in Massachusetts. Given that glyphosate is typically a terrestrially applied herbicide, EPA's risk assessment (EPA 2015a) found potential direct toxicity effects to fish to be limited. Further, as noted in this review, it is uncertain whether glyphosate aquatic exposure due to spray drift or other means would elicit the types of sublethal effects to T&E species as observed in other species in laboratory tests (see the Section "Effects on Fish"). EPA's BE (2021) concluded that glyphosate is likely to adversely affect 179 of the 190 listed fish species nationally. Glyphosate was also determined likely to adversely affect the critical habitats for 105 of the 107 fish species where critical habitats have been determined (EPA 2021).

- *Amphibians*. There are no currently listed endangered amphibians in Massachusetts; but there are three threatened species and two species of special concern. As noted by Bach et al. (2016) and Wang et al. (2019c), the potential direct effects to amphibians (i.e., lethal and sublethal) of glyphosate are relatively unknown; and limited research indicates that there may be sublethal effects on amphibian growth and development due to glyphosate exposure. Indirect effects of glyphosate (i.e., effects on food and habitat) on amphibians may occur in cases where their habitat is adjacent to fields or other habitats (e.g., transportation rights of way) where GBFs are applied. For example, all five MA-listed salamander species may potentially be at risk where glyphosate is applied, but it is uncertain whether amphibians would be adversely affected at glyphosate concentrations expected based on labeled uses. EPA's BE (2021) concluded that glyphosate is likely to adversely affect all (36) of the nationally listed amphibian species. Glyphosate was also determined to likely adversely affect critical habitats for 25 amphibian species where critical habitats have been determined (EPA 2021).

- *Reptiles*. There are currently eight endangered reptile species listed in Massachusetts, five threatened species, and three species of special concern. Five of these species are sea turtles and are considered to have little or no exposure to glyphosate, which is one of the reasons why EPA (2021) considered sea turtles to not be at risk. The other eleven species (terrestrial species) may have limited exposure directly to glyphosate but could experience indirect effects due to decreased potential food resources (e.g., plants or lower trophic level animals used for food) and habitat impacts (e.g., plants used for shelter). Like amphibians, reptile species—including those listed by MA (e.g., copperhead, timber rattlesnake, eastern hog-nosed snake, Blanding's turtle, wood turtle, and bog turtle)—most at risk to glyphosate exposure would be those that preferentially inhabit areas adjacent to fields or other habitats (e.g., transportation rights of way) where glyphosate is applied. EPA's BE (2021) concluded that glyphosate is likely to adversely affect 33 of the 47 nationally listed reptile species. Glyphosate was also determined likely to adversely affect critical habitat for 10 of 16 reptile species where critical habitats have been determined (EPA 2021).
- *Birds*. There are currently nine endangered species of birds listed in Massachusetts, seven threatened species, and fourteen species of special concern. EPA (2021) indicated that the listed bird species may have limited direct exposure to glyphosate but could experience indirect effects due to decreased potential food resources (e.g., plants or lower trophic-level animals used for food) and due to impacts on habitat (e.g., plants used for shelter). Bird species (including those listed by MA) most at risk to glyphosate exposure would be those that preferentially inhabit areas adjacent to fields or other habitats (e.g., transportation rights of way) where glyphosate is applied. EPA's BE (2021) concluded that glyphosate is likely to adversely affect both (a) 88 of the 108 nationally listed bird species and (b) critical habitats for 30 of 31 bird species where critical habitats have been determined.
- *Invertebrates and Insects*. There are currently 32 endangered invertebrate or insect species listed in Massachusetts, 25 threatened species, and 42 species of special concern. The listed species may have limited direct exposure to glyphosate but could experience indirect effects via the same mechanisms identified in the previous paragraphs: impacts to food resources and habitats. Invertebrate and insect species most at risk to glyphosate exposure are those that preferentially inhabit areas adjacent to fields or other locations (e.g., transportation rights of way) where glyphosate is applied. EPA's BE concluded that glyphosate will be likely to adversely affect 185 of 207 nationally listed aquatic invertebrate species and 140 of 160 listed terrestrial invertebrate species (EPA 2021). Glyphosate was also determined likely to adversely affect critical habitats for 68 of 71 aquatic invertebrate species and 38 of 49 terrestrial invertebrate species where critical habitats have been determined (EPA 2021). Indirect effects due to changes in critical habitat may include a decrease in preferred pollinator plant species or specific plants required for insect survival (e.g., monarch butterfly dependence on milkweed) that are outcompeted by glyphosate-resistant weed species.
- *Plants*. There are currently 153 endangered plant species listed in Massachusetts, 66 threatened species, and 40 species of special concern. The listed plant species may have high exposure directly to glyphosate and are also vulnerable to glyphosate exposure. Plant species (including those listed by MA) most at risk from glyphosate exposure would be those that preferentially inhabit areas adjacent to fields or other locations (e.g., transportation rights of way) where glyphosate is applied. EPA's BE (2021) concluded that glyphosate is likely to adversely affect 940 of 948 listed plant species. Glyphosate was also determined likely to adversely affect critical habitats for 456 of 460 plant species where critical habitats have been determined (EPA 2021). Effects on critical habitat of listed plant species may include increased abundance and distribution of glyphosate-resistant weed species that may outcompete T&E plant species in their preferred habitat.

2.6.4 Summary of Environmental Effects

This technical review of glyphosate's environmental effects suggests that, in general, the lack of acute effects on invertebrates and vertebrates, identified in EPA's 2015 preliminary risk assessment and in the 2021 draft EU environmental assessment, are supported by recent peer-reviewed literature. Also, EPA's BE (EPA 2021) indicates that glyphosate may impact federally listed plant species (as well as most other plant species, as expected) that may result in indirect effects on animals in terms of habitat quality or quantity and food availability. Data gaps

identified by both the EPA and the EU environmental assessments, which were the focus of this review, are related to sublethal or long-term toxicity effects on terrestrial and aquatic animals, as well as indirect effects on their habitat, particularly habitat of pollinators such as bees and monarch butterflies.

This review identified very few recent peer-reviewed studies that met the search criteria and definitively filled data gaps with respect to long-term toxicity effects or indirect effects of glyphosate on animals. One potentially important effect, not highlighted in EPA's preliminary risk assessment but the subject of many recent research articles in this review, concerns the effects of glyphosate on the soil microbiome (for which there is currently mixed evidence regarding the effects of glyphosate) and the effects on symbiotic microflora in bees. Glyphosate is known to have effects on many species of bacteria and fungi because bacteria and fungi are susceptible to the same mechanism of action for glyphosate as in plants (blocking the shikimate pathway for protein synthesis); however, as noted previously, more in-depth studies are needed to understand glyphosate's antimicrobial effects.

Several studies in this review indicate that glyphosate applied at realistic concentrations affects the composition of bacteria and fungi species in soil, particularly under anaerobic conditions, which may result in deleterious effects on plant growth and perhaps certain soil invertebrate species. Based on current information compiled in this review, it remains uncertain as to the range of glyphosate's antimicrobial effects and the extent to which those result in adverse effects on plants and soil biota.

The effects of glyphosate on the microflora of bees are being investigated by many researchers. Several publications compiled in this review reported direct effects of glyphosate on the microflora in bees resulting in indirect effects on bees, such as lower resistance to disease and other immune deficiencies. Thus far, it is uncertain as to the extent to which glyphosate's indirect effects on bee populations or hives are due symbiotic microflora effects and lower disease resistance or other factors. Another factor perhaps related to glyphosate, which could also be responsible for reported declines in some bee populations, may be related to a decrease in local food resources because of an increase in glyphosate-resistant weeds that are a poorer source of honey (see below).

Indirect effects of glyphosate on monarch butterflies due to direct effects on milkweed, which is required for monarch survival, is also an intensively researched area due to the widespread use of glyphosate-containing herbicides. While current research compiled in this review suggests that potential indirect effects of glyphosate on monarch butterfly populations are plausible, a study definitively demonstrating these indirect effects has not been conducted due to the complex relationship between monarch population survival and other environmental factors, such as habitat loss in their wintering sites and climate change (e.g., increased air temperatures, more frequent severe weather events).

As noted in EPA's preliminary risk assessment (EPA 2015), glyphosate is acutely toxic to nearly all plant species unless the plant is genetically modified to be glyphosate tolerant or naturally becomes resistant. EPA's BE (EPA 2021) further indicated that federally listed plant species are likely to be impacted by glyphosate and it identified potential indirect effects of glyphosate on federally listed species ranging from plants to mammals and birds. Some of the species examined by EPA occur in Massachusetts, and many species of concern in Massachusetts may have similar habitat features as some federally listed species. Therefore, many state-listed species that are exposed to glyphosate due to overspray, spray drift, or runoff, may also be subject to indirect effects of glyphosate. As noted previously, the "likely to adversely affect" designation is based on highly conservative analyses, and this designation does not mean that entire species are in jeopardy or that critical habitats are being adversely modified. Rather, this determination is primarily intended to identify the subset of issues to be further evaluated by EPA in consultation with other agencies (e.g., National Marine Fisheries Service, National Forest Service) for a Biological Opinion on whether potential effects to individuals might negatively impact populations or the entire species or adversely impact a critical impact and whether risk mitigation measures are warranted. Thus, the process initiated by the BE on risks to threatened and endangered species is ongoing and more detailed information on potential impacts will become available in the forthcoming Biological Opinion.

Several peer-reviewed publications compiled in this review noted that weed resistance to glyphosate and their resulting proliferation in field edge habitats, along with acute effects on non-resistant plant species that serve as important food and habitat for species of concern (and other species not currently federally or state-listed in Massachusetts), may be an ecologically important indirect effect of glyphosate. As with other indirect effects identified in this review, indirect effects of observed increases in glyphosate-resistant weed species and associated

local declines in native plant species abundance in certain habitats (e.g., agricultural field edges, rights of way) on listed and unlisted species is a critical issue to many researchers and conservation organizations.

This review found that while glyphosate may have sublethal toxic effects on biota besides plants at maximum prescribed application rates for some uses, GBFs are often reported to be more toxic than glyphosate alone, based on testing with the same glyphosate concentration used in the formulation or by itself. Many references compiled in this review reported toxicity of certain surfactants (e.g., POEA) used in GBFs were responsible for at least some of the reported toxicity effects; and several publications (e.g., Rodríguez-Gil et al. 2021) attribute considerably greater toxicity to GBFs that contain POEA when compared to glyphosate technical. EPA also addressed the incremental toxicity associated with POEA surfactant in GBFs by in its ecological risk assessment (EPA 2015a). We note that many organizations and researchers in the U.S., Europe, and elsewhere recommend greater scrutiny of the toxicity of "inert" ingredients or co-formulants used in GBFs, as well as greater protection of biota from potential indirect effects of glyphosate.

3.0 Findings on Glyphosate Alternatives

ERG's scope of work requires a review of key glyphosate alternatives. The Phase One report identified four categories of alternatives that ERG proposed evaluating: chemical methods (defined and discussed in Section 3.1), mechanical methods (Section 3.2), physical methods (Section 3.3), and biological methods (Section 3.4). The Phase One report also listed individual alternatives within these categories. This section presents ERG's review of these alternatives, plus others identified during the Phase Two research.

Applicators consider a range of factors when selecting methods to control vegetation. These include effectiveness at controlling the target organism (both over the near term and long term), potential environmental and human health impacts, ease of implementation, the number of applications required, public acceptance, applicable regulations and restrictions, and cost. For a given herbicide control method, these factors may differ from one application to the next. For instance, a control method that is feasible for spot treatment of weeds near an ornamental garden might not be feasible for controlling weeds at a large farm or along hundreds of miles of highways. That said, ERG was not charged with determining whether glyphosate alternatives should be used in the first place and, if so, for which applications. ERG was also not charged with conducting cost-benefit analyses of every alternative. Rather, this section reviews relevant characteristics of the categories of alternatives, based on the information ERG compiled in Phase Two.

The review of glyphosate's human health and environmental impacts in Section 2.5 and Section 2.6, respectively, was based entirely on key assessments and peer-reviewed publications. This review of alternatives considers the following information sources:

- EPA pesticide registration findings for chemical alternatives only.
- Research on glyphosate alternatives for Massachusetts herbicide applications (e.g., Barker and Prostak 2008; Barker and Prostak 2009; Mattei 2022).
- More than 50 Vegetation Management Plans and Yearly Operational Plans for Massachusetts rights of way. These were prepared pursuant to 333 CMR 11.00 and are posted to the Executive Office of Energy and Environmental Affairs website.
- Public input received during Phase Two. While this input included a range of opinions on glyphosate and its alternatives (e.g., glyphosate should be banned, some alternatives are feasible for certain uses, some alternatives are more harmful than glyphosate, no alternatives are feasible for certain uses), this section only considers observations documenting specific experiences with alternatives. Appendix B of the final report will present a compilation of all public input received.
- Interviews that ERG conducted with government agency representatives from other Northeast states.
- Selected other publications that describe or evaluate glyphosate alternatives for U.S. locations outside of Massachusetts (e.g., Chiotti et al. 2020; DRISI 2019; Neal and Senesac 2022).

As Section 2 notes, glyphosate-containing herbicides have been registered in Massachusetts for more than 100 different weeds (or weed pests) and vegetation management and for more than 100 different application sites.

Evaluating the thousands of combinations of pests, application sites, and alternatives is a monumental task, and no researcher has systematically evaluated all these combinations; however, research has been conducted on certain categories of applications, including for applications that account for the greatest proportion of glyphosate used in Massachusetts, per the statistics shown in Section 2.3. To the extent supporting information is available, this section reviews information on glyphosate alternatives for the following application categories: rights of way, lawn care, consumer use, corn agriculture, and invasive species management.

3.1 Chemical Methods

ERG considered multiple chemical herbicides as glyphosate alternatives. These alternatives exhibit a range of properties relevant to weed and vegetation control (e.g., systemic vs. contact herbicides; selective vs. non-selective herbicides; pre-emergent vs. post-emergent herbicides). As noted in the Phase One report, the chemical herbicide alternatives fall into two groups.

- EPA-registered herbicides. Table 1 in the Phase One report listed 22 EPA-registered active ingredients that others have identified as candidate glyphosate herbicide alternatives. These were identified by reviewing multiple reports investigating alternatives (Barker and Prostak 2008; 2009; Chiotti et al. 2020; MassDOT 2021; Neal and Senesac 2022; UMass CAFE 2020). ERG's Phase Two research identified three additional herbicide alternatives. Two herbicide active ingredients (fosamine and paclobutrazol) were identified from ERG's review of more than 50 Yearly Operational Plans that municipalities, utilities, rail operators, and other parties submitted to MDAR pursuant to a Massachusetts rights of way vegetation management regulation (333 CMR 11.00). The other active ingredient (mesotrione) was identified in the public input as an herbicide used for weed control in lawn care.

  Table 10 in this report lists the active ingredients in EPA-registered herbicides that this scientific review considered further; however, just because this table lists potential alternatives does not mean they have been demonstrated to serve as effective glyphosate substitutes in Massachusetts or elsewhere. The public input received in Phase Two did not allow for narrowing down this list. ERG notes that one stakeholder requested removing dithiopyr, fluazifop-p-butyl, and indaziflam from this scientific review because they are fluorinated active ingredients, and therefore considered to be per- and polyfluoroalkyl substances (PFAS). Although these substances include between one and five fluorine atoms in their molecular structures, ERG retained these alternatives in this scientific review because a universally accepted list of PFAS chemicals is not available. The stakeholder's concern is included here for reference.

  ERG's first step in evaluating the glyphosate alternatives was to compile selected government agency ratings of potential impacts. Many such rating schemes are available, and Table 10 displays the acute toxicity categories assigned by EPA and a water quality indicator assigned by the University of California Statewide Integrated Pest Management Program. The EPA indicators are based on acute toxicity studies of oral exposure, inhalation exposure, dermal exposure, eye irritation, and skin irritation; the University of California indicator pertains to water-quality risks due to runoff. As the table shows, the glyphosate alternatives include some with ratings suggesting higher acute toxicity and others with lower acute toxicity; a range of ratings is also shown for runoff risk potential. Although these indicators do not characterize the full range of pesticides' human health and environmental impacts, they suggest that the pesticides identified as alternatives are not necessarily less toxic or harmful to human health and the environment than glyphosate—a similar point was raised in the public input (i.e., that some alternatives may be more harmful than glyphosate).

- Minimum risk pesticides. The other chemical alternatives to glyphosate-containing products are those that meet the criteria for minimum risk pesticides, and therefore EPA does not register them under the Federal Insecticide, Fungicide, and Rodenticide Act. To be eligible for this designation, the products must contain active ingredients and inert ingredients from lists of substances developed by EPA (EPA 2015c; 2016) and meet additional criteria for labeling, health claims, and other factors. Examples of active ingredients for minimum risk pesticides include citric acid, clove oil, coconut oil, corn gluten meal, garlic oil, and lauryl sulfate (EPA 2015c). Formulations containing acetic acid at concentrations up to eight percent are also eligible to be minimum risk pesticides, provided the other applicability criteria are met. ERG notes that public input on Phase Two recommended that this scientific review consider minimum risk

pesticides, organic pesticides, and natural pesticides, which we assume collectively refers to the minimum risk pesticides described here.

The following discussion reviews available information on chemical herbicide alternatives for several categories of uses:

- Vegetation management in rights of way. As Section 2.3 noted, one of the largest glyphosate uses in the northeast is for managing vegetation in rights of way, whether for roadways, railways, or utilities. This use generally involves removal of all vegetation.

  In 2008, researchers from the University of Massachusetts Transportation Center studied the viability of alternative chemical herbicides for vegetation management in roadside rights of way managed by the Massachusetts Department of Transportation (MassDOT) (Barker and Prostak 2008). The two-year study evaluated effectiveness in greenhouses, alongside highways, and in field plots on a research farm. The study considered multiple chemical herbicide alternatives to glyphosate, including clove oil, pelargonic acid, citric-acetic acid, and limonene. While some of these alternatives were effective at short-term vegetation suppression, the efficacy lasted only three to six weeks after which another application was necessary. The researchers noted that the material costs for alternatives were higher than for conventional herbicides—and in some cases "substantially so." These material costs do not include the costs associated with multiple applications to achieve effectiveness over the longer term. The researchers ultimately concluded that the alternative chemical herbicides evaluated were "less effective and more costly" than conventional herbicides (e.g., glyphosate).

  For further insight into vegetation management at roadside rights of way, ERG reviewed several yearly operational plans submitted by municipalities. These differ from the MassDOT example in terms of scale. Specifically, MassDOT reports managing vegetation alongside more than 3,000 miles of roadways throughout the Commonwealth (MassDOT 2021); the city of Quincy, just as one example, manages closer to 200 centerline miles of roadway, all within a much more localized area (City of Quincy 2021). Most roadside vegetation management plans that ERG reviewed included a section titled "justification of herbicide use." In many plans, this section lists several areas for which chemical herbicides are necessary (e.g., areas that are difficult or impossible for mowers to reach, areas that would pose a safety hazard to workers using mowers, etc.). These plans further note poison ivy as a special case for which herbicide use is justified, largely because of the health risks posed by mowing and the need to kill the entire plant (see Section 3.2 for further information). Finally, some plans indicate that chemical herbicides are the treatment method of choice for invasive plants.

  ERG also reviewed a dozen yearly operational plans and vegetation management plans for railroads. According to multiple plans, federal law (49 CFR Part 213, Section 37) requires that vegetation on railroad property on or near railbeds be managed for various reasons (e.g., fire prevention, enhanced visibility, safety). Several plans that ERG reviewed have herbicide justification statements such as: "The railroad vegetation management program requires the use of specific herbicides because, based on railroad experience, this is the only means proven to manage vegetation adequately" (Wood 2021). A vegetation management plan prepared on behalf of seven railroads in Massachusetts identifies the three herbicide active ingredients that railroads have primarily used over the last twenty years: glyphosate, metsulfuron-methyl, and sulfometuron-methyl (TEC Associates 2021). Glyphosate's effectiveness as a post-emergence, non-selective herbicide is noted; and limitations are cited for the other two active ingredients (e.g., metsulfuron-methyl has limited effectiveness on grasses). The plan notes that imazapyr has more recently been used for managing vegetation in railroad rights of way, but herbicides containing this active ingredient cannot be applied in consecutive years in Massachusetts, necessitating the use of glyphosate and the other two active ingredients in years when imazapyr cannot be used (TEC Associates 2021). A common theme expressed across the plans is that "…on the railroad roadbed, no suitable alternative to herbicide vegetation control is currently available" (TEC Associates 2021).

- Professional lawncare and landscaping. As Table 1 illustrates, glyphosate used by landscape contractors, lawncare operators, and those who manage turf at schools, parks, and other settings accounts for a considerable portion of annual non-agricultural glyphosate use in the northeast. Unlike rights of way

applications, which involve systematic removal of all vegetation from a given area, lawncare and landscaping uses tend to be more targeted. They generally aim to control a range of weeds, while not harming established turf and plants. The targeted weeds include grasses, broadleaves, and sedges, which can be annual or perennial.

ERG did not locate peer-reviewed publications evaluating the effectiveness of chemical alternative herbicides for professional lawncare and landscaping applications in Massachusetts. However, some academics have posted articles to their websites on this topic. For instance, researchers from the Extension Turf Program with University of Massachusetts Amherst's Center for Agriculture, Food, and the Environment (CAFE) posted an online article about glyphosate alternatives (UMass CAFE 2000). The article compares weed-control effectiveness between glyphosate, glufosinate, diquat, pelargonic acid, and minimum risk herbicides (e.g., acetic acid, clove oil, citric acid, d-limonene). The comparison is based on "known data" but further details on the comparative methodology are not presented. It evaluated control effectiveness for four different categories of weeds: summer annual weeds, winter annual weeds, simple or solitary perennial weeds, and creeping or spreading annual weeds. For all four categories, glyphosate was reported as offering "excellent control," and the alternatives' control ratings ranged from "good" to "very poor." Further, among the herbicides considered, glyphosate was the only option rated as appropriate for complete turf renovation. The authors acknowledge that their findings are based strictly on weed-control effectiveness—not on consideration of other factors (e.g., potential impacts to human health and the environment).

Additionally, weed-science researchers from Cornell University and North Carolina State University published an article on glyphosate alternatives for landscaping (Neal and Senesac 2022). The authors note that viability of alternatives depends on specific details of the weed-control scenario. For instance, they indicate that other chemical herbicides and minimum risk pesticides can be effective at controlling seedling annual broadleaf weeds; however, these methods are less effective for controlling established annual weeds, perennial weeds, and grasses. The authors ultimately conclude: "Although there are effective alternatives to glyphosate, each of these alternatives will be, in some way, less effective, less convenient, and/or more expensive." They also acknowledge that: "Landscape weed control without glyphosate is certainly possible but will require more planning, careful consideration of alternative treatments, more frequent site visits, and higher costs. But it can be done" (Neal and Senesac 2022).

Finally, a team of researchers affiliated with the University of California investigated the effectiveness of glyphosate and multiple alternatives on urban landscaping applications (Reiter and Windbiel-Rojas 2020). The study investigated herbicide performance on swatches of lawn at a golf club considering two glyphosate formulations and several organic herbicides (e.g., citric acid and clove oil, limonene, caprylic acid, acetic acid, pelargonic acid, ammoniated soap of fatty acids). All products tested had evidence of "significant injury" to weeds shortly after application; however, weed growth eventually resumed a few weeks later in areas treated with organic products, and the glyphosate-treated areas continued to show "significant injury" for the entire six-week observation period. The authors concluded: "Switching from glyphosate-containing products to organic herbicides will require a reallocation of resources for more frequent applications at higher product volumes. Resources will include costs for labor involved in more frequent applications, possible increased costs in purchasing additional personal protective equipment, training for handling more acutely toxic products, and higher product costs" (Reiter and Windbiel-Rojas 2020).

The public input provided further context on glyphosate use for lawncare and landscaping. One stakeholder who represents lawncare professionals indicated that landscapers select herbicides that are most effective with the least harmful negative impacts. This individual explained that lawncare professionals routinely use non-glyphosate herbicides and tend to use glyphosate as an alternative when these other options fail. Finally, the individual expressed a willingness to adopt effective organic weed control products but added that experience to date suggests that organic alternatives are more expensive and do not provide satisfactory results. On the other hand, as evidence of the feasibility of chemical herbicide alternatives, another stakeholder indicated that "many municipalities" only use organic weed control methods in landscaping; the stakeholder noted the town of Brookline as an example.

TABLE 10. TOXICITY AND OTHER HAZARD RATINGS FOR SELECTED GLYPHOSATE ALTERNATIVES

Herbicide	EPA Ratings					California Water Quality Runoff Risk Rating
	Acute Oral Toxicity Group	Acute Inhalation Toxicity Group	Acute Dermal Toxicity Group	Primary Eye Irritation Rating	Primary Skin Irritation Rating	
Glyphosate	III	III	III	III	IV	Moderate
2,4-D	III	III	III	I	IV	Low
Aminopyralid compounds	IV	IV	IV	IV	IV	–
Chlorsulfuron	IV	IV	III	IV	IV	Low
Clethodim	III	III	IV	III	I	Low
Clopyralid compounds	IV	III	IV	I	III	–
Diquat compounds	III	III	II	II	IV	Moderate
Dithiopyr	IV	IV	IV	IV	II	High
Fluazifop-P-butyl	III	III	III	IV	IV	High
Fosamine	IV	IV	II	IV	IV	–
Glufosinate compounds	III	III	III	III	IV	Low
Imazapyr compounds	IV	II	III	I	IV	Low
Imazethapyr compounds	IV	III	III	III	IV	–
Indaziflam	III	IV	III	IV	IV	–
Isoxaben	IV	IV	II	II	III	High
Mesotrione	IV	IV	III	IV	IV	–
Metsulfuron, methyl-	IV	IV	III	III	III	–
Oryzalin	IV	III	III	III	III	High
Paclobutrazol	III	II	III	II	III	–
Pendimethalin	III	IV	IV	III	IV	Very high
Prodiamine	IV	III	III	III	IV	High
Sethoxydim	III	III	III	IV	IV	Moderate
Simazine	IV	III	III	IV	IV	–
Sulfometuron-methyl	IV	IV	III	III	IV	–
Triclopyr compounds	III	IV	III	I	IV	Moderate

Notes: Toxicity category I is for the most toxic substances, and toxicity category IV is for substances that are not acutely toxic. Category I substances have a signal word of "Danger" on the label, category II is "Warning," category III is "Caution," and category IV does not have a signal word.

EPA ratings are taken from pesticide registration eligibility decisions, EPA fact sheets, National Pesticide Information Center fact sheets, and other resources.

California ratings are from the University of California Statewide Integrated Pest Management Program's pesticide active ingredients database entries, available at https://ipm.ucanr.edu/PMG/menu.pesticides.php; "—" entries do not have runoff water ratings posted to that website.

The Phase One report identified caprylic acid, d-limonene, and pelargonic acid as potential alternatives. They are not included in the table because EPA has designated them as inert ingredients; these substances do not have toxicity ratings.

- Consumer use. According to EPA estimates, the largest non-agricultural use of glyphosate in the Northeast is consumer applications (see Table 1). EPA has also published estimates of the most-used herbicide active ingredients for household lawn and garden purposes in the United States (see Table 11); multiple herbicide products containing these ingredients are available to consumers in Massachusetts. ERG did not locate a study that compared performance of these active ingredients in residential applications. To comment on the alternatives, ERG considered basic descriptors of herbicide action, recalling that glyphosate is a non-selective, systemic herbicide.

TABLE 11. MOST COMMONLY USED HERBICIDES NATIONWIDE IN THE HOUSEHOLD LAWN AND GARDEN SECTOR, 2012

Rank	Active Ingredient	Estimated Annual Usage
1	2,4-D	7,000,000 – 9,000,000 lbs.
2	Glyphosate	4,000,000 – 6,000,000 lbs.
3	Methylchlorophenoxypropionic acid (MCPP)	2,000,000 – 4,000,000 lbs.
4	Pendimethalin	2,000,000 – 4,000,000 lbs.
5	Dicamba	1,000,000 – 3,000,000 lbs.
6	2-Methyl-4-chlorophenoxyacetic acid (MCPA)	1,000,000 – 3,000,000 lbs.

Source: EPA, 2017

The non-glyphosate alternatives in Table 11 have documented herbicidal activity for weeds, but none is non-selective and systemic; therefore, none of them will control the full range of weeds that glyphosate does. For example, EPA pesticide fact sheets note that: 2,4-D, dicamba, MCPA, and MCPP are selective for broadleaf weeds, and pendimethalin is selective for both broadleaf weeds and grassy weeds; these do not show the broader range of non-selective effectiveness that glyphosate offers. It should be noted that some of the herbicides listed in Table 11 have formulations that are "State Restricted Use" and therefore are not direct glyphosate substitutes for certain users.

ERG searched pesticide distributor websites to identify herbicide products commercially marketed as non-selective that do not contain glyphosate. The active ingredients in these products include mixtures containing bromacil, diuron, glufosinate, diquat, imazapyr, imazethapyr, pelargonic acid, prometon, tebuthiuron, triclopyr, and others (DIY Pest Control 2023). Therefore, various non-selective herbicide alternatives are available for consumer uses, but ERG did not locate studies comparing the effectiveness of these alternatives for residential applications.

The information reviewed indicates that non-selective chemical herbicide alternatives to glyphosate exist for consumer uses, but a systematic review of relative effectiveness specifically for the consumer market was not identified. The public comments offered no further details on consumer uses, beyond one commenter noting that "numerous home gardeners use no chemical pesticides at all."

- Agriculture. As noted previously, agricultural glyphosate usage in Massachusetts accounts for a relatively small fraction of the statewide total usage—a factor of approximately 30 less than non-agricultural usage. This review focuses on glyphosate alternatives for corn farming because it accounts for an estimated 88 percent of the statewide agricultural glyphosate usage (see Section 2.3). ERG identified various references that present glyphosate alternatives for corn farmers; however, we focused on Massachusetts-specific references because the average farm size in the state (69 acres) is considerably smaller than the national average (445 acres) (USDA 2022).

  The principal reference the ERG team considered for glyphosate alternatives for corn farms is the 2023-2024 *New England Vegetable Management Guide*, which is a resource with detailed crop-specific information on pest management and various other topics (New England VMG 2023). The *Guide* promotes integrated pest management approaches that consider a broad range of management practices to reduce the need for herbicide use. It also specifies options for different classes of herbicides, including non-selective herbicides, pre-emergent herbicides, post-emergent herbicides, and perennial weed control. For all but one class, the *Guide* lists multiple chemical herbicides and provides guidance on their use (e.g., application rates, mixing tips, when to apply). As the exception, glyphosate is the only chemical

herbicide option listed for perennial weed control. The *Guide* does not present information on relative effectiveness across herbicide options, but integrated pest management guides from other states do (e.g., UGA 2022).

The public input did not include specific recommendations for chemical alternatives to glyphosate for agriculture. However, one stakeholder noted that many commercial farmers in Massachusetts exclusively use minimum risk pesticides for weed control.

- Invasive species management. During phone interviews with state agency officials and through the public input process, ERG learned that glyphosate and other chemical herbicides are used to control invasive species in Northeast states, and these applications are sometimes necessary to protect endangered species, threatened species, and sensitive habitats. In these applications, which are typically targeted use of glyphosate in well-defined areas, we further learned that rapid and complete control is essential. Public input also indicated that there can be risks associated with not using glyphosate in these cases (i.e., the threat from invasive species can outweigh impacts associated with targeted herbicide applications).

  During its Phase Two research, ERG located only one resource that evaluated the effectiveness of non-glyphosate alternatives for invasive plant management. The resource, a webpage published by the University of Massachusetts Extension Turf Program, characterized effectiveness of six pesticides: glyphosate, glufosinate, diquat dibromide, pelargonic acid, and "non-chemicals" (a term that the webpage used to refer to selected minimum risk pesticides). For invasive plant management, glyphosate was the only option rated as "excellent." The alternatives had ratings for controlling seedlings of "good" and "good to fair" and ratings of either "poor" or "very poor" otherwise (UMass CAFE 2020). The methodology used to assign these ratings, however, is not described. Finally, ERG notes that the only Northeast state that currently bans glyphosate use on all state property (New York) has a provision in the requirement that permits glyphosate use for control of invasive species.

Two general observations not specific to an application are noted regarding chemical herbicide alternatives to glyphosate. First, Los Angeles County recently commissioned an expert committee to evaluate a range of glyphosate alternatives. The committee reported that chemical methods "are amongst the most effective, safest, and least expensive methods available" (Chiotti et al. 2020). The committee report notes that effective use of chemical methods requires training and knowledge of herbicide application concepts and that chemical methods are not the most effective alternative in all cases. Second, during the public input process, a stakeholder indicated that many risks associated with chemical herbicide use can be "managed through judicious application (e.g., timing, methods, equipment, etc.)."

3.2 Mechanical Methods

The Phase One report indicates that mechanical methods use mechanical devices to control weeds and other vegetation. Examples include mowing, flaming or steaming weeds, killing them with foam, and tilling soil. Various parties have reported on the advantages and disadvantages of these options (e.g., Chiotti et al. 2020; MassDOT 2021). ERG has compiled those observations here. The review is organized around the different types of mechanical methods. Currently, some methods are not commonly used in Massachusetts, but are reviewed nonetheless for greater context on alternatives.

Mowing is perhaps the most familiar mechanical alternative to glyphosate and other chemical herbicide use. Nearly every right of way yearly optional plan (YOP) that ERG reviewed identified mowing as an herbicide alternative. This alternative can include ride-on mowers, push mowers, and others.

Advantages of mowing as a glyphosate alternative include:

- Does not require specialized licensing.
- Like glyphosate, mowing is non-selective. It removes all vegetation above the height of cut.
- Allows for rapid removal of low-growing vegetation over large stretches of land (e.g., roadsides).
- Effective at removing dense vegetation, including vegetation that might pose a risk for wildfire.

Disadvantages of mowing include:

- Does not remove vegetation beneath the height of cut, including roots of weeds and plants. As a result, many weeds and plants will continue to grow, and target areas will need to be mowed periodically to achieve long-term effectiveness.
- Not a practical option for steep slopes, narrow medians, under guardrails, around signposts, and in otherwise inaccessible areas (although trimming with hand-held tools can be used in these circumstances).
- Can reduce the effectiveness of subsequent herbicide applications, possibly because weeds and grasses in mowed areas have less surface area for herbicides to contact.
- Can contribute to weed propagation when mowing weeds with mature seeds.
- Does not kill entire poison ivy plants and mowing action can release the toxic oil (urushiol) that affects humans.
- Can lead to noise complaints.

Flaming or steaming weeds and other vegetation has also been investigated as a glyphosate alternative. Flame devices typically burn butane or propane, and heat from the torch essentially boils weeds. Steam devices direct steam or superheated water at a target to scald weeds and their root systems. Temperatures for these instruments can reach 2,000 ^{o}F for flame and 265 ^{o}F for steam or superheated water. For both techniques, hand-held and tractor-based options are available. Steam equipment can apply chemical herbicides and foams in addition to steam or superheated water. In Massachusetts, thermal techniques are reportedly being used on farms but not for highway right-of-way clearing; research studies have investigated the effectiveness of these techniques at roadside and agricultural fields in the state.

Advantages of flaming and steaming as a glyphosate alternative include:

- Achieves results shortly after treatment.
- Effective on small woody plants that might be difficult to control by mowing.
- Like glyphosate, flaming and steaming is non-selective; it controls all post-emergent vegetation.
- Applications can be highly targeted, with less concern (when compared to sprayed herbicides) about impacts to non-target species.
- Steaming (without chemical additives) has no concerns about toxicity to the applicator or chemical contamination to surface water or groundwater.
- Avoids disturbing soil.

Disadvantages of flaming and steaming include:

- Might need multiple applications (or use of multiple methods) to achieve long-term effectiveness, particularly for year-round plants or grasses.
- Thermal techniques control above-ground vegetation but do not control roots; effectiveness is limited for perennial weeds with extensive root systems.
- Flame and steam techniques raise concerns of burn injury to applicators and handling flammable substances (for flame devices) introduces safety concerns.
- Flame techniques increase risk for fires, including wildfires caused by igniting brush or mulch and structural fires if used near buildings. Permits may be required from local fire officials before use.
- Although flaming methods are intended to control plants and weeds via their high temperatures and not through catching them on fire, inadvertent burning of vegetation with poison ivy would present a health hazard to applicators and bystanders, because inhaling smoke from these fires can cause health effects (e.g., lung irritation) (NIOSH 2010).

ERG's Phase Two research identified infrared heating control as another category of thermal treatment. The infrared devices have internal flames that heat a surface on the equipment, which may be ceramic or metal. That heat then radiates to the weed and plant targets. Researchers in Oregon and Vermont have investigated the effectiveness of infrared heating for weed control, but ERG found no evidence of this technology being used in Massachusetts.

Foaming, as the term implies, involves spreading foam over the target area. Foams that are chemical herbicide products are not discussed here, because they fall under the "chemical methods" category (see Section 3.1). This section considers foam applied in tandem with steam. Though designs vary, one technology relies on a biodegradable foam, and the intended function of the foam is to trap the steam on its target, which essentially increases the contact time for the steam (Barker and Prostak 2008). MassDOT reported on a comparative study of weed control effectiveness for steam technology versus steam with foam technology and found the foam-based system to be only "slightly more effective" (Barker and Prostak 2008). The same advantages and disadvantages for the steaming method apply here, except the foam-based systems have an added disadvantage of potential environmental effects of the foam constituents (even though they are reported as being biodegradable).

Tilling is a common practice used for many purposes in agriculture; it involves turning over and breaking up soil. Tilling controls weeds by uprooting, cutting, and burying them. Across all rights of way YOPs that ERG reviewed, tilling was not mentioned as an herbicide alternative, and tilling does not appear to be a weed-control method of choice for most non-agricultural applications.

Advantages of tilling as a glyphosate alternative include:

- Effective in controlling deep-rooted perennial weeds.
- A viable option at locations that can be accessed by tractors, rototillers, and other equipment.
- Allows for efficient weed control over broad areas (e.g., farmlands).

Disadvantages of tilling include:

- Typically, must be used in conjunction with other weed-control methods (e.g., herbicides and mulching) to achieve desired effectiveness.
- Can contribute to weed growth by improving soil conditions and by spreading portions of weeds (e.g., rhizomes) that can reestablish and grow.
- Can lead to soil erosion.

3.3 Physical Methods

The Phase One report describes physical methods as options for controlling weeds manually, whether by removing them from the soil (e.g., hand-picking weeds, hoeing weeds, etc.) or by applying mulch, weed mats, or other materials to suppress their growth. This section considers the same primary sources of information (e.g., Chiotti et al. 2020; MassDOT 2021) when commenting on the strengths and limitations of these physical methods.

Manual removal, as the name implies, entails taking weeds out of the soil, whether by hand or through use of hand-held tools such as hoes. Although it can theoretically be used for any weed-control application, manual removal is more commonplace for certain non-agricultural applications.

Advantages of manual weed removal as a glyphosate alternative include:

- A viable alternative at locations where herbicide use is prohibited and where mowers cannot access.
- Used in applications for invasive species control and in sensitive habitats.
- Trained workers or volunteers can identify and remove weed species of concern while avoiding desired plants.
- Does not involve use of chemical herbicides.
- Limited environmental impacts.

Disadvantages of manual removal include:

- The most labor-intensive alternative and generally not feasible for large areas, like transportation corridors and rail and utility rights of way.
- Not effective at controlling perennial weeds, weeds with deep underground root systems, or densely vegetated areas.
- Health and safety concerns for certain site characteristics, including presence of vehicular traffic, poison ivy, mosquitos, and ticks.

Mulching is the covering of a land surface with another material. In the context of weed control, mulching is typically used to prevent new weeds from growing (as opposed to controlling established weeds). It does so by preventing sunlight from reaching weed seeds already on soil, acting as a barrier for weed growth, and preventing new seeds from depositing on soil. Various types of materials are used as mulch, including bark, wood chips, straw, and compost. Mulch can be applied manually or by using mulch spreaders and other equipment. In a recent Massachusetts research project on mulching, an application thickness of two to three inches was used.

Advantages of mulching as a glyphosate alternative include:

- When used following a flame-control treatment in the research project noted above, one layer of mulching provided "excellent suppression" of weeds and other vegetation for an entire growing season, without the need for herbicides; and the effectiveness continued into a second growing season (Barker and Prostak 2008).
- Provides an option for using only natural, biodegradable materials with no synthetic chemicals.
- Water conservation is a co-benefit.
- Can be used in areas that mowers cannot readily access, such as around signposts and beneath guardrails.
- Helps to reduce erosion.

Disadvantages of mulching include:

- Typically requires some form of pre-treatment of existing weeds.
- Transporting and applying large quantities of mulch can be both logistically challenging and labor intensive.
- Weeds can grow through mulch layers, especially when mulch layers thin or wear away over time.
- Can contribute to weed growth if mulch material contains weed seeds.
- Improperly applied mulch material can wash away during rainstorms.

Weed mats are layers placed on top of soil. They are made from plastic, cardboard, and other materials. Weed mats block sunlight and act as a barrier to weed growth, but they allow moisture and air to pass through. Installers may opt to apply chemical herbicide beneath the mats for increased weed-control effectiveness. Weed mats are available in a range of materials, thicknesses, and durability.

Advantages of weed mats as a glyphosate alternative include:

- High degree of weed control.
- Can be implemented without using chemical herbicides.
- Highly durable options can last longer than 15 years and be maintenance-free.

Disadvantages of weed mats include:

- Rated "unacceptable" for ease of implementation and startup and ongoing costs in the Los Angeles County evaluation of glyphosate alternatives (Chiotti et al. 2020).
- Breaches that form in the mats compromise the control efficiency.

ERG's Phase Two research of rights of way YOPs identified other weed-control methods that, like weed mats, involve physical barriers to weed growth. These included sealing cracks, repaving roads, and performing other roadway repairs. These options are used to prevent future weed growth and do not control existing weeds (although weeds would be cleared by some other method before making the noted surface improvements).

3.4 Biological Methods

The Phase One report describes biological methods as the use of other organisms to remove weeds or inhibit their growth. These include the use of herbivores (e.g., sheep, goats, cattle) or insects to consume weeds, pathogens to kill or weaken weeds, and other plants (e.g., clover) to compete with weeds. In its review of glyphosate alternatives, MassDOT noted "few instances" of biological methods for controlling weeds for roadside applications (Barker and Prostak 2008). Moreover, the Los Angeles County review of glyphosate alternatives concluded that biological methods are "infeasible as a replacement for glyphosate because of their limited applicability" (Chiotti et al. 2020). Nonetheless, due to reports of grazing being used for weed control in Massachusetts, particularly in pasture lands (UMass not dated), this section reviews grazing as an alternative.

Advantages of grazing as a glyphosate alternative include:

- Can be implemented without using chemical herbicides.
- Useful for sites (e.g., sloped surfaces) that cannot be readily controlled with mowing and other alternatives.
- Effective control of annual plants and weeds.
- A feasible alternative in locations such as pastures, where animals can be fenced in.

Disadvantages of grazing include:

- Controls above-ground vegetation but does not remove roots that can lead to regrowth.
- Animals might graze on desired vegetation in addition to controlling weeds.
- Not feasible for roadside applications given animal-vehicle collision hazards.
- Animals' hoof action and feces might cause environmental damage.

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5.0 Abbreviations Used in the Report

A	acre
AChE	acetylcholinesterase
ADI	Acceptable Daily Intake
AE	acid equivalents
AGG	Assessment Group on Glyphosate
AHS	Agricultural Health Safety
AMPA	aminomethylphosphonic acid
APVMA	Australian Pesticide and Veterinary Medicines Authority
aRfD	acute reference dose
ASD	autism spectrum disorder
ATSDR	Agency for Toxic Substances and Disease Registry
BE	biological evaluation
CBI	confidential business information
CDC	Centers for Disease Control and Prevention
CI	confidence interval
CKDu	chronic kidney disease of unknown etiology
DCR	Department of Conservation and Recreation
DLBCL	diffuse large B-cell lymphoma
EC	European Commission
ECHA	European Chemicals Agency
EDSP	Endocrine Disruptor Screening Program
EFSA	European Food Safety Authority
EPA	U.S. Environmental Protection Agency
EPSPS	enolpyruvyl-shikimate phosphate synthesis
ERG	Eastern Research Group, Inc.
ESSPS	enolpyruvyl-skikimate-3-p
EU	European Union
FAO	Food and Agriculture Organization
FSCJ	Food Safety Commission of Japan
FSH	follicle-stimulating hormone
GAP	good agricultural practices
GBF	glyphosate-based formulations

GnRH	gonadotropin-releasing hormone
HQ	hazard quotients
IARC	International Agency for Research on Cancer
ID	Interim Decision
LHRH	luteinizing hormone-releasing hormone
LOAEL	lowest observed adverse effect level
MassDEP	Massachusetts Department of Environmental Protection
MCPA	2-methyl-4-chlorophenoxyacetic
MCPP	methylchlorophenoxypropionic
MDAR	Massachusetts Department of Agricultural Resources
MM	multiple myeloma
MRL	minimal risk level
NAPP	North American Pooled Project
NCI	National Cancer Institute
NGO	non-governmental organization
NHANES	National Health and Nutrition Examination Survey
NHL	non-Hodgkin lymphoma
NIEHS	National Institute of Environmental Health Sciences
NIOSH	National Institute for Occupational Safety and Health
NOAEL	no observed adverse effect level
NRDC	Natural Resources Defense Council
NSRL	No Significant Risk Level
NTP	National Toxicology Program
OEHHA	California Office of Environmental Health Hazard Assessment
OPP	Office of Pesticide Programs
OR	odds ratio
PFAS	polyfluoroalkyl substances
PMRA	Canada's Pest Management Regulatory Agency
POEA	polyethoxylated tallowamine
PPE	personal protective equipment
RED	Reregistration Eligibility Decision
RR	relative risk
T&E	Threatened and Endangered
TSH	thyroid stimulating hormone

UMass	University of Massachusetts
USDA	U.S. Department of Agriculture
USFS	U.S. Forest Service
USGS	U.S. Geological Survey
WHO	World Health Organization
YOP	Yearly Operational Plan

Appendix A. Public Comments Received on the Draft Phase 2 Report

Comment from David Saltmiras, Senior Principal Toxicologist, Bayer Crop Science.

Comment from Richard Lawlor, CGCS retired.

To Ann Lowery, Assistant Commissioner
via email to Jesse Grant (Jesse.A.Grant@mass.gov), Program Coordinator
MassDEP Bureau of Policy & Planning
Boston, MA

July 19, 2024

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RE: Glyphosate Scientific Review Phase 2 Report

Dear Assistant Commissioner Lowery:

Thank you for the opportunity to provide feedback on the Glyphosate Scientific Review Phase 2 Report prepared for the Massachusetts Glyphosate Commission (hereinafter, Commission).

The Phase 2 report clearly demonstrates the dominant conclusion, regarding the safety of glyphosate determined by the EPA, is that there are no identified cancer or non-cancer human health risks associated with registered uses of glyphosate. Additionally, assessments by various agencies such as ATSDR, OEHHA, EFSA/ECHA, FAO/WHO, PMRA, FSCJ, and APVMA have concluded that glyphosate does not pose significant risks to human health in terms of carcinogenicity, genotoxicity, and other adverse health effects. These determinations collectively indicate a consistent finding across multiple regulatory bodies that glyphosate, when used according to specified guidelines, does not present unacceptable risks to human health.

To continue its support of the Commission, Bayer would like to provide the following corrections and interpretations as the Phase 2 report is finalized.

GENERAL COMMENTS:

Executive Summary comments

1. The Center for Disease Control and Prevention urine monitoring detections are mentioned in terms of numbers of detections, but the context for human health should be noted for human exposure levels vs the US EPA chronic Refence Dose (cRfD). Human exposures are orders of magnitude lower than the US EPA acceptable human daily exposures.
2. It would be appropriate to note that IARC does not review the regulatory studies that are required by US EPA or any other regulatory or authoritative body to conduct robust human health risk assessments. IARC uses literature and secondary sources of information. While IARC selectively cites the US EPA review, they come to an opposite conclusion to the Agency, despite having not reviewed the underpinning data supporting the US EPA conclusion that glyphosate does not present a cancer risk.
3. The Phase 2 report refers to the pending assessment of glyphosate in Europe. Please note, this review is now complete, and glyphosate is reapproved for a further 10 years in the European Union. The European Commission, all four Rapporteur Member States, and the European Chemicals Agency all concluded that glyphosate does not present a cancer risk to humans.
4. In the European Union (EU), the European Food Safety Authority (EFSA) oversees the comprehensive hazard characterization and risk assessment of assigned European Member State regulatory authorities, usually one *Rapporteur* and one *Co-Rapporteur*. In the recent review and December 2023 10-year renewal of glyphosate, four Rapporteurs, France, Hungary, the Netherlands, and Sweden, together reviewed multiple regulatory toxicology data packages from multiple EU registrants and the scientific literature. This review commenced in June 2020. Following public consultation of their draft Renewal Assessment Report, public comments were collected and reviewed, then a revised draft RAR was prepared and submitted to EFSA for a detailed scientific peer review, which was finalized in 2023. In parallel, the European Chemicals Agency (ECHA) Risk Assessment Committee (RAC), accessed the same information to conduct a thorough hazard characterization for the purpose of classification and labeling.

HUMAN HEALTH COMMENTS:

2.5.1 General Considerations for the Scientific Review of Human Health Effects

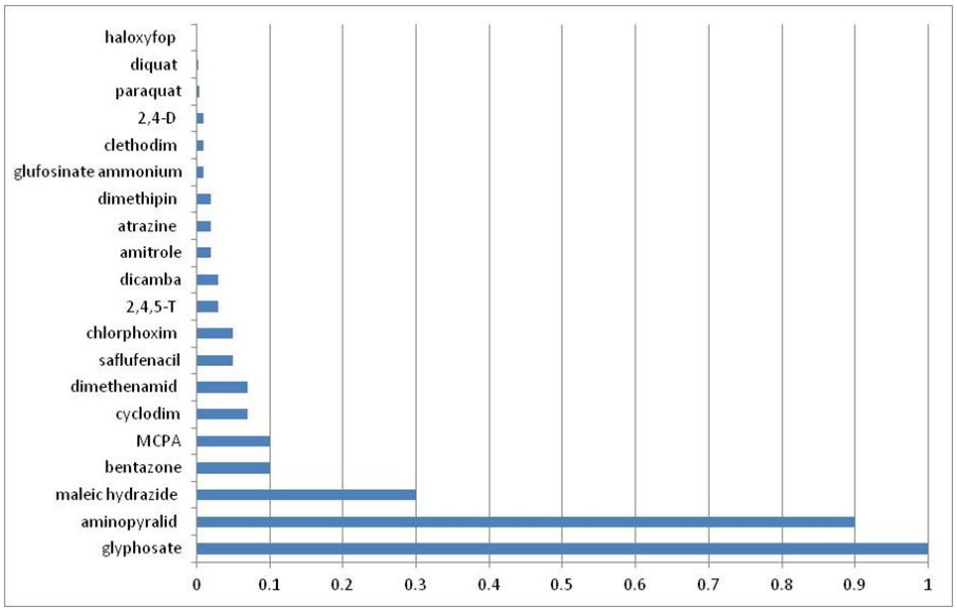
Under *Differences between glyphosate technical and glyphosate-based formulations* it is important to note

- The first sentence mentions *additional ingredients, some of which are known to be toxic*. This is a vague statement which contradicts fundamental tenets in the disciplines of toxicology and human health risk assessment (i) toxicity is a function of dose-response; (ii) route of exposure plays an important role in toxicity; and (iii) it is important to know which endpoint(s) may be in question when determining something is "toxic".
- Glyphosate Based Herbicides (GBHs) typically consist of glyphosate salt, surfactant(s), and water. Surfactants are in general known to be eye and potentially skin irritants, and as such, surfactants are more acutely toxic than glyphosate salts. Both glyphosate and surfactants in general have been determined to not present either genotoxic risk or cancer risk to humans. This is reflected in authoritative agency assessments where primary toxicology study reports and data sets are reviewed first-hand. It is, however, important to note IARC does not review toxicology study reports, only secondary sources of these study reports, and often comes to contrary conclusions to the reviewers of those primary toxicology study reports.
- The US EPA evaluates each formulation to ensure human safe use, and that the toxicological profile of each individual ingredient must first be approved by the US EPA for use in pesticide products. For example, the US EPA reviewed the class of surfactants which include POEA [Federal Register /Vol. 74, No. 115 /Wednesday, June 17, 2009], noting an acute reference dose (RfD) of 0.72 mg/kg/day, and chronic RfD of 0.15 mg/kg day. Thus, POEA is "more toxic" than glyphosate, in that there is no US EPA acute RfD for glyphosate, and the current chronic RfD is for glyphosate is 1.0 mg/kg/day. However, both POEA and glyphosate are non-genotoxic and non-carcinogenic.
- Under "Metabolites of glyphosate", please note that aminomethylphosphonic acid (AMPA) is found in the environment from non-glyphosate sources, as a degradant of phosphonate detergents. Only a small amount of glyphosate may be metabolized

in the gastro-intestinal (GI) tract, which is excreted in feces as AMPA, and this metabolism is believed to be due to microbial metabolism in the GI tract. Systemic glyphosate is not metabolized to AMPA to any significant extent, and unmetabolized glyphosate is rapidly filtered by the kidneys and excreted in urine. AMPA also has a comparable toxicological profile to glyphosate.

- Under “Effects levels”, it is important to note that US EPA chronic RfD and foreign/international authority Acceptable Daily Intake (ADI) levels are based on the No Observed Adverse Effect Levels (NOAELs), and that glyphosate NOAEL values, and thus ADI values are typically higher (i.e. “less toxic”) than other active ingredients. See figure below.

World Health Organization Herbicide ADI values (mg/kg bw/day) up to 2013[1]



[1]Saltmiras, DA, Farmer, DR, Mehrsheikh, A and Bleeke, MS (2015). *24 Glyphosate: The Fate and Toxicology of a Herbicidal Amino Acid Derivative*, book chapter in Amino Acids in Higher Plants Editor J.P.F. D’Mello. Publisher CAB International, Figure 24.4., p. 476

2.5.2 Assessments Issues by Government Agencies and International Bodies

Updates for paragraph 4 on page 13

- Regulatory authority assessments from South America have been inadvertently omitted.
    - Brazilian Health Surveillance Agency (ANVISA) completed their multi-year reassessment of glyphosate concluded in March 2019, established an ADI of 0.5 mg/kg bw/day, and reaffirmed that glyphosate is not mutagenic, not teratogenic, and not carcinogenic.
    - The National Health Institute of Columbia issued a GBH non-carcinogen classification determination in January 2024.
- New Zealand Environmental Protection Authority (NZ EPA) recently denied an application requesting a reevaluation of glyphosate, stating "grounds do not exist for the reassessment of glyphosate and glyphosate-containing substances". NZ EPA staff determined that based on extensive and comprehensive reviews by other international regulators, "the risks associated with the use of glyphosate had not significantly changed" since the previous NZ EPA assessment. APP204718-Grounds-for-reassessment-Decision.pdf (epa.govt.nz)

Table 2. Human Health Assessments Published by Government Agencies and International Bodies

- IARC: It is important to note that the data IARC relied upon for this determination of "sufficient evidence in animals" was based on limited second-hand information from regulatory or other authoritative reviews, and IARC came to the opposite conclusion of these regulatory and other reviews. IARC did not review the toxicology study reports or underlaying pathology reports that the cited authorities evaluated to conclude that glyphosate does not cause cancer in animals.
- IARC: Regarding epidemiological papers of pooled analyses and meta-analyses, please consider these in context of the recent paper by Acquavella (2023) [Epidemiologic studies of glyphosate and non-Hodgkin's lymphoma: A review with consideration of exposure frequency, systemic dose, and study quality, *Global Epidemiology* 5 (2023) 100101, https://doi.org/10.1016/j.gloepi.2023.100101].
- NTP: Please also note Chan and Mahler (1992) also conducted Ames and in vivo micronucleus assays and found that glyphosate was not genotoxic. NTP more recently evaluated glyphosate and

GBHs for the potential to cause oxidative stress. Data were presented at SOT in 2019, concluding that glyphosate and its formulations do not induce DNA damage and oxidative stress (Rice et al., 2019, provided).

- OEHHA: Please note that the OEHHA basis for setting a NSRL is solely based on IARC's evaluation of hemangiosarcoma in male mice in one of five available chronic carcinogenicity studies in mice. IARC cited JMPR (2006) published hemangiosarcoma data for one study. While JMPR reviewed the actual study report and pathology report, IARC did not review these reports and came to a different conclusion than JMPR.
- EFSA/ECHA: Please note that the EFSA and ECHA reviews are independent of each other, and each separately evaluated the relevant toxicology data sets and scientific literature. EFSA concluded that glyphosate does not pose a cancer risk to humans. ECHA concluded that glyphosate should not be labeled as a carcinogen.

2.5.4 Cancer Effects

- Page 21, end of top partial paragraph, please add additional paper for context on De Roos et al. (2022), with references, and subsequent details from follow up paper [Acquavella J (2003), Epidemiologic studies of glyphosate and non-Hodgkin's lymphoma: A review with consideration of exposure frequency, systemic dose, and study quality. *Global Epidemiology* 5 (2023) 100101. https://doi.org/10.1016/j.gloepi.2023.100101
- Reiterating the above comments, please note what IARC relied upon for this determination of "sufficient evidence in animals". IARC's assessment was based on limited second-hand information from regulatory or other authoritative reviews, and IARC came to the opposite conclusion of these regulatory and other reviews. IARC did not review the toxicology study reports or underlaying pathology reports that the cited authorities evaluated to conclude that glyphosate does not cause cancer in animals.
- Page 21 under short paragraph under assessments concluding glyphosate is non-carcinogenic, please include Brazil (ANVISA) and The National Health Institute of Columbia.

- Page 21 under review of human data for NHL and MM, first bullet Zhang et al. (2019a), please add a sub-bullet that includes information on the June 20, 2024, decision by Judge Chhabria (Federal judge presiding over the Roundup MDL) to exclude the testimony and opinions of Dr. Zhang. The Judge characterized the Zhang et al. (2019a) meta-analysis as 'junk science' with 'deep methodological problems'. https://law.justia.com/cases/federal/district-courts/california/candce/3:2020cv03719/360503/88/
- Page 23, please add two final bullet points under *Review of Human Data for NHL and MM*, summarizing both Acquavella (2023) and Sorahan (2015) [Sorahan T (2015). Multiple Myeloma and Glyphosate Use: A Re-Analysis of US Agricultural Health Study (AHS) Data. *Int. J. Environ. Res. Public Health* 2015, *12,* 1548-1559; doi:10.3390/ijerph120201548 http://www.unboundmedicine.com/medline/citation/25635915/Multiple_Myeloma_and_Glyphosate_Use:_A_Re-Analysis_of_US_Agricultural_Health_Study_(AHS)_Data]
- Page 24, the claims in Portier (2020) were each specifically addressed in detail in Volume 1 of the European Renewal Assessment Report of glyphosate, which concluded that glyphosate is not carcinogenic.
- Page 25, under Review of recent literature (2019-223) on genotoxic, epigenetic, and other effects on DNA, please consider opening the paragraph with some discussion on the lack of biological plausibility for such effects, given that glyphosate is not systemically metabolized and thus not activated to form reactive intermediates to initiate such alleged effects.

2.5.5 Reproductive Effects

- Page 27 please note that ECHA evaluations of classification and labelling for reproductive effects also includes assessment of developmental toxicities, which concluded that classification for such effects is not warranted.
- Please correct the following statement on page 27, *Other assessments listed in Table 2 did not address reproductive toxicity.* The following all evaluated potential to cause reproductive effects.
    ..1. 2017 EFSA and ECHA evaluations

..2. 2022 and 2023 ECHA and ECHA evaluations
..3. NTP Chan and Mahler (2012)
..4. PMRA of Health Canada

2.5.8 Developmental Effects

- Please correct the following statement on page 38, “Other assessments listed in Table 2 did not address developmental toxicity”. The following all evaluated potential to cause reproductive effects.
  ..1. 2017 ECHA evaluation
  ..2. 2022 and 2023 ECHA and ECHA evaluations

2.5.9 Renal Toxicity

- Please note, scientific literature and toxicology data sets are reviewed by all regulatory agencies and other authoritative groups include assessment of renal effects. Thus, all regulators and JMPR in Table 2 did appropriately evaluate renal effects.

Other Human Health Effects

- Immunological Effects: US EPA, Canadian PMRA, and EFSA in Europe all evaluated immunotoxicity, including a GLP compliant in vivo immunotoxicity assay and determined that glyphosate was not toxic to the immune system.

ENVIRONMENTAL IMPACT COMMENTS:

2.6.1.1 EPA Registration Review – Preliminary Exposure Risk Assessment for Glyphosate and Its Salts (2015)

- Page 53; In section 2.6.1.1, discussion of EPAs Preliminary Ecological Risk Assessment is presented, the document states that that the Agency review incorporated ‘... available exposure and effects data up through 2015, and that the most current modelling and risk assessment methodologies were conducted’. It should be acknowledged that relative to the 2015 review, agency approaches being used in the Preliminary Ecological Risk Assessments conducted by the agency today (2024) differ. For example, GENEEC and TerrPLANT are no longer used by the agency to determine exposure levels in water and effect levels on plants. Instead, PWC

(Pesticide in Water Calculator) and PAT (Plant Assessment Tool) are used today.

- Furthermore, concerning EPAs 2015 Registration Review, - also on page 53, reference is made to three application rates (3.75, 8 and 40 lb/A). These reflect the maximum single application rates taken from a Joint Glyphosate Task Force Uses matrix, which the agency used to inform on application rates to be used in exposure modelling in the Preliminary Ecological Risk Assessment. The Uses matrix was collated by registrants to understand the broad range of uses and rates across all glyphosate registrants in the USA. In fact, the applied use rates in the exposure modelling are highly conservative as most glyphosate maximum single application rates applied in the US are much lower. This point was raised during the round of public commenting on the Preliminary Ecological Risk Assessment (2015) and the Biological Evaluation (2021) by registrants and grower groups.
- Concerning the agencies Biological Evaluation (2021) on page 55, the report states that that EPA's conclusion that technical glyphosate is 'practically acutely non-toxic to terrestrial and aquatic animals', ... was based on a review of **all registered labels** for herbicide products containing glyphosate. In fact, although some glyphosate labels are mentioned in the Biological Evaluation (2021), the exposure modelling used to inform wildlife exposure in the field, was based on use rates from the Joint Glyphosate Task Force Uses matrix as discussed in the previous bullet point, that is not considered a federal action / label.

2.6.1.2 EPA Biological Evaluation (2021)

- Page 56, It would be appropriate to mention The US Fish & Wildlife Service as a key agency involved in the production of the Biological Opinion.

2.6.1.3 European Union Glyphosate Environmental Assessments

- On page 58, reference is made to the EFSA and AGG having classified glyphosate as Chronic Category 2 and should be labelled as "toxic to aquatic life with long lasting effects" based on the toxicity data considered at the time and the fact that glyphosate does not rapidly degrade. However, it is the European Chemicals Agency

(ECHA) who are responsible for concluding on the classification of glyphosate. The ECHA conducted an evaluation that considers the data evaluated by EFSA and the AGG. In fact, ECHA included additional 'non-regulatory' data (not considered by EFSA and the AGG) from the public domain to support their final conclusion.

2.6.2.5 Pollinator Insects: Bees

- On page 64, reference is made to a study by Zioga et al. (2022) assessing the effects of glyphosate-based formulations on pollinator plants oilseed rape and blackberry, commonly found near edge habitat of agricultural field. Authors of this work found glyphosate in blackberry samples taken 2-7 days after application, although the magnitude of residues is not stated. In study work conducted by *Thompson et al. (2012), to evaluate the potential for toxicity to developing honey-bee larvae and pupae of glyphosate when fed at rates up to 301 mg a.e./kg dite, directly to honey-bee colonies (exposed via sugar solution placed directly inside the colonies), the authors determined residues of glyphosate in developing larvae of up to 53 mg a.e./kg on day 4 following exposure. This reduced to 4.1 mg a.e./kg by day 7. This level of exposure resulted in no significant adverse treatment related effects being observed in any of the exposed colonies. (*Thompson et al. (2012) Evaluating exposure and potential effects on honeybee brood (Apis mellifera) development using glyphosate as an example. Integr Environ Assess Manag 10(3): 463-70. doi:10.1002/ieam.1529).

As the Commission closes this scientific review, it is important to highlight the importance of incorporating a Weight of Evidence (WoE) approach to assess the quality of the studies used in the scientific decision-making process. New and existing studies, including literature, must be evaluated against their reliability, relevance, and consistency. Studies which are invalidated or contain low quality data must be removed from consideration. This Phase 2 document highlights a number of studies which have not been measured against this WoE approach and could erode the systematic scientific based decision-making process. To support this scientific review by the Massachusetts Glyphosate Commission, Bayer would support the transparent review of documents provided in order to evaluate in a WoE approach.

Sincerely,

David Saltmiras

David Saltmiras, PhD, DABT
Senior Principal Toxicologist
Bayer Crop Science

Table 1. Regulator conclusions on animal carcinogenicity studies with glyphosate

Year	Reviewing Body	Tumors related to treatment with glyphosate?			
		Knezevich & Hogan (1983) 24-months mouse	Atkinson (1993) 104 weeks mouse	Lankas (1981) 26-months rat	Stout & Ruecker (1990) 24-months rat
1987	WHO/JMPR	No	-	No	-
1991	US EPA Cancer Classification	No	-	No	No
1991	Canada PMRA	No	-	No	No
1993	US EPA RED	No	-	No	No
1994	WHO/IPCS	No	-	No	No
1999	Japan FSC	No	-	No	No
2000	FAO Specifications	No	-	No	No
2002**	EU Annex 1 Listing	No	No	No	No
2004**	WHO/FAO JMPR	No	No	No	No
2005	WHO/Water Sanitation Health	No	-	No	No
2007	California EPA OEHHA	No	No	No	No
2008	US EPA Effects Determination	No	-	No	-
2012	US EPA Human Health RA	No	-	No	No
2013	Australia APVMA	No	No	No	No
2015	IARC*	Yes	Yes	Yes	Yes
2015**	EU AIR2 (BfR & EFSA)	No	No	No	No
2015**	Canada PMRA PRVD	No	No	No	No
2016**	WHO/FAO JMPR	No	No	No	No
2017**	US EPA Revised Issue Paper	No	No	No	No
2017**	EFSA RAC 40	No	No	No	No
2017**	Canada Final RVD	No	No	No	No
2019**	Brazil ANVISA	No	No	No	No
2022**	EFSA RAC 61	No	No	No	No
2023**	EU AIR5 (AGG & EFSA)	No	No	No	No

* IARC cited secondary data sources only... the above [contrary] EPA and JMPR reviews of the primary data

** Reviews included up to 8 rat & 5 mouse chronic/carcinogenicity studies as well as published scientific literature

John Wilhelmi

From: Grant, Jesse A (DEP) <Jesse.A.Grant@mass.gov>
Sent: Tuesday, July 23, 2024 8:12 AM
To: Lowery, Ann (DEP)
Subject: Fw: MA DEP Approves Glyphosate Registration
Attachments: Glyphosate%20Scientific%20Review%20-%20Phase%20Two%20Report%20%286-18-2024%29%20For%20Public%20Comment.pdf

Forwarded from Rick Lawlor:

Good Day Jesse,

I wanted to explain why I copied you on my email to our elected officials. I have spoken with a number of "you" over the years about helping those of us who value your hard work and service to MA residents. However when some residents/legislators get restless with bad information, the legislative process in general or the position of our paid experts, business professionals need your help in defending our work which is licensed by federal and state legislation. This is especially true for us "down on old Cape Cod" and other hotbeds of home rule petitions (for everything) in this state.

One of the things that I constantly say to the nay sayers, "Why yes, I awoke this morning with the purpose of seeing how many people I could harm in the course of my work today." We public servants (Town of Yarmouth 17 years), need to defend our work and make our taxpayers understand that we are the consummate professionals, guided by laws and mandates diligent to the taxpayer expenses and safety.

Keep Up the Good Worrk,

Richard T. Lawlor, CGCS retired
11 Heather Hill Road
Sandwich, MA 02563
508-681-9568

From: rick lawlor <rickstang302@yahoo.com>
Sent: Saturday, July 20, 2024 9:35 AM
To: Moran Susan (SEN) <susan.moran@masenate.gov>; Xiarhos Steven - Rep. (HOU) <steven.xiarhos@mahouse.gov>; Julian Cyr <julian.cyr@masenate.gov>; Dylan.Fernandes@mahouse.gov <dylan.fernandes@mahouse.gov>; Marc Pacheco <marc.pacheco@masenate.gov>
Cc: Christopher Lauzon <votelauzon@gmail.com>; Grant, Jesse A (DEP) <Jesse.A.Grant@mass.gov>
Subject: MA DEP Approves Glyphosate Registration

The US EPA did as well! I find it troubling, to say the least, that our elected LOCAL officials continually denounce the decisions of our federal and state service divisions to promote fear among their constituents in order to develop a dependent voter base. This is morally wrong. This is where the trust in OUR system dissolves.

As you are all well aware Barnstable County also approved this chemical for use on the public right-of-ways AND local conservation commissions (following the science and recommendations of environmental engineering firms) are pleading for its use to remedy the invasive species overtaking our shore lines.

I am providing you this copy of the finding because you can't deny its existence. Glyphosate Highlights: General Use classification; mode of action is plant specific; product is strongly bound to the soil (it doesn't leach); the label (which is the law) does GREAT job of directing for safe use; and the EPA has invoked compliance with the Endangered Species Act for registration approval. MA ranks 50 out of 52 states for total product use (kind of like our gun problem).

Representative Dylan Fernandes spoke about a moral obligation to residents, it is my hope that you all embody that sentiment and stop traveling offshore for your science and solutions. Stand behind OUR investment and the work of OUR state services. Follow your oath.

Richard T. Lawlor
11 Heather Hill Road
Sandwich, MA 02563