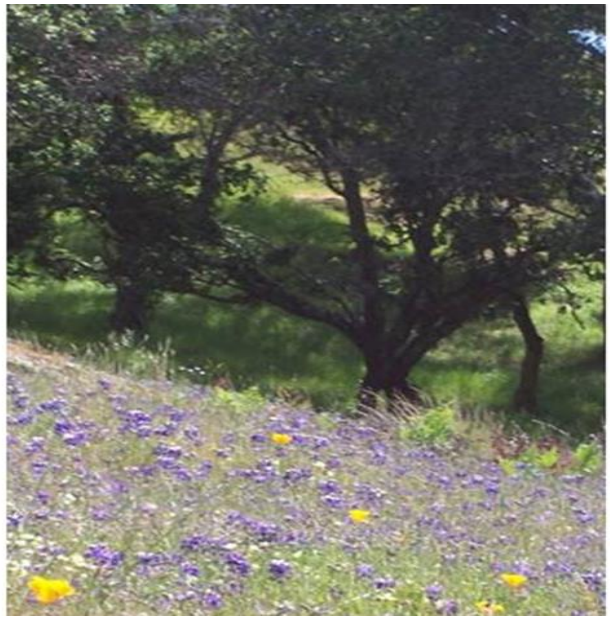




Marin Municipal Water District

Final Program Environmental Impact Report for the Biodiversity, Fire, and Fuels Integrated Plan

State Clearinghouse No. 2017012007

October 2019

PANORAMA
ENVIRONMENTAL, INC.
717 Market Street Suite 650 San Francisco, CA 94103 650-373-1200 **www.panoramaenv.com**

Marin Municipal Water District

Final Program Environmental Impact Report for the Biodiversity, Fire, and Fuels Integrated Plan

October 2019

Prepared for:
Marin Municipal Water District
220 Nellen Avenue
Corte Madera, CA 94925

Prepared by:
Panorama Environmental, Inc.
717 Market Street, Suite 650
San Francisco, CA 94103
650-373-1200
info@panoramaenv.com

PANORAMA
ENVIRONMENTAL, INC.

TABLE OF CONTENTS

List of Appendices

List of Tables

This page is intentionally left blank.

ACRONYMS, SYMBOLS, AND ABBREVIATIONS

BFFIP	Biodiversity, Fire, and Fuels Integrated Plan
CCR	California Code of Regulations
CDFW	California Department of Fish and Wildlife
CNPS	California Native Plant Society
CRPR	California Rare Plant Ranking
District	Marin Municipal Water District
EIR	Environmental Impact Report
GHG	greenhouse gas
GIS	Geographic Information Systems
IARC	International Agency for Research on Cancer
IPM	Integrated Pest Management
MCL	Marin Conservation League
MM	Mitigation Measure
NPIC	National Pesticide Information Center
PG&E	Pacific Gas and Electric Company
PRC	Public Resources Code
SOD	Sudden Oak Death
USEPA	U.S. Environmental Protection Agency
VMP	Vegetation Management Plan
WAFRZ	Wide Area Fuel Reduction Zone
WPHIP	Wildfire Protection and Habitat Improvement Plan
WUI	wildland-urban interface

This page is intentionally left blank.

1 INTRODUCTION

1.1 PURPOSE OF THE FINAL EIR

Marin Municipal Water District (District) proposes to implement the Biodiversity, Fire, and Fuels Integrated Plan ("BFFIP" or "proposed project"). This Program Environmental Impact Report (EIR) has been prepared in accordance with the California Environmental Quality Act (CEQA) (Public Resources Code [PRC] § 21000 *et seq.*) and the amended Guidelines for the Implementation of CEQA (CEQA Guidelines) (14 California Code of Regulations [CCR] § 15000 *et seq.*) and provides an assessment of the potentially significant environmental effects of the proposed BFFIP.

The District is the "lead agency" for the BFFIP evaluated in this Final Program EIR and the Board of Directors is responsible for the certification of this Final Program EIR as adequate and complete. The District has prepared this Final Program EIR to:

- Inform the general public and decision makers about the nature of the BFFIP, potentially significant environmental effects, feasible mitigation measures to avoid or mitigate those effects, and reasonable and feasible alternatives to the proposed project;
- Enable the District to consider the environmental consequences of approving the BFFIP; and
- Satisfy CEQA requirements.

In accordance with the CEQA Guidelines, after completion of the Draft Program EIR, the District is required to consult with and obtain comments from affected public agencies, and to provide the public with an opportunity to comment on the Draft Program EIR. The District is then required to respond to significant environmental issues raised in the review and consultation process (CEQA Section 15132).

As described in CEQA and the CEQA Guidelines, public agencies are charged with the duty to avoid or substantially lessen significant environmental effects of proposed projects, where feasible. A public agency is obligated to balance the proposed project's significant effects on the environment with its benefits, including economic, social, technological, legal, and other benefits. The Program EIR is an informational document that, as required by CEQA, (1) assesses the potentially significant environmental effects of the proposed plan, including cumulative impacts, (2) identifies feasible mitigation measures to avoid or substantially reduce significant impacts, (3) identifies any significant and unavoidable adverse impacts that cannot be mitigated to less than significant levels, and (4) evaluates a range of reasonable alternatives to the proposed project, including the No Project Alternative, that would eliminate or substantially reduce any significant adverse environmental effects of the proposed project.

The CEQA lead agency is required to consider the information in the EIR, along with any other relevant information in the administrative record, in making its decision on a proposed project. Although the EIR does not determine the ultimate decision that will be made regarding implementation of the proposed project, CEQA requires the District to consider the information in the EIR and make findings regarding each significant effect identified in the EIR before it can approve the proposed project. The Board of Directors would need to certify this Final Program EIR prior to adopting the BFFIP. The Board of Directors is required to consider the information in the Program EIR, along with any other relevant information in the administrative record, in making its decision on the BFFIP.

1.2 SUMMARY OF THE PROPOSED PROJECT

The purpose of the BFFIP is, in a large part, to identify the tools and actions the District can take to reduce fuel loads and fire risks and improve ecosystem health. The BFFIP identifies 27 specific actions that are designed to achieve the goals of minimizing the risk from wildfires, preserving and enhancing existing significant biological resources, and allowing for an adaptive framework for the periodic review and revision of BFFIP implementation in response to changing conditions and improved knowledge.

Of these 27 actions, 19 are considered administrative and would include inventorying and monitoring resources, partner collaboration, and planning for various District activities. The remaining eight management actions include vegetation management in the field through the use of hand tools and mechanical equipment to establish and maintain fuelbreaks and defensible spaces; to remove invasive plant species; and to improve and restore native ecosystems on watershed lands.

Herbicides are not included as part of the plan. All work would be performed using manual and mechanical tools and equipment, and prescribed burning.

1.3 ENVIRONMENTAL REVIEW PROCESS

The Draft Program EIR was prepared to analyze the environmental impacts of the proposed project. The Draft Program EIR considered the proposed project and alternatives that would reduce or avoid significant environmental impacts. The Draft Program EIR was circulated to affected public agencies and interested parties for a 90-day review period from March 21, 2019 to June 19, 2019. Comments on the Draft Program EIR were to be submitted in writing by no later than 5:00 pm on June 19, 2019.

In conformance with CEQA Guidelines Section 15151, EIRs should be prepared with a sufficient degree of analysis to provide decision-makers with information that enables them to decide on the project and considers environmental consequences. The Final Program EIR is required to examine mitigation measures and alternatives to the project intended to reduce or eliminate significant environmental impacts.

The Final Program EIR will also be available for review at the following locations:

- San Rafael Public Library
- Mill Valley Public Library
- Bolinas Library
- Stinson Beach Library
- Larkspur Library
- Fairfax Library
- Corte Madera Library
- Town of San Anselmo Public Library
- Marin Municipal Water District Main Office
- Marin Municipal Water District Project Website: www.marinwater.org/bffip

In accordance with the CEQA guidelines, the Final Program EIR will be made available to the public and commenting agencies a minimum of 10 days prior to the EIR certification hearing.

1.4 REPORT ORGANIZATION

This document is organized as follows:

- **Chapter 1: Introduction**. This chapter includes a discussion of the purpose and organization of the Final Program EIR.
- **Chapter 2: Responses to Comments**. This chapter contains copies of comments received during the public review period and responses to those comments. Each comment letter is coded. Each comment is bracketed in the margin of the letter and assigned a secondary, comment-specific number. For example, the first comment in the letter from the California Native Plant Society is A1-1. Each comment letter is followed by a response corresponding to the bracketed comment. Master responses are also provided on topics raised by several commenters.
- **Chapter 3: Revisions to Text of Draft EIR**. This chapter presents corrections or clarifications to the Draft Program EIR based on comments received. The text changes have not resulted in significant new information with respect to the proposed project, including any new potentially significant environmental impacts that cannot be mitigated to less than significant, or in any new mitigation measures. Corrections to the text and tables of the Draft EIR are contained in this chapter. <u>Underlined text</u> represents language that has been added to the Draft Program EIR; text with ~~strikethrough~~ has been deleted from the Draft Program EIR.
- **Chapter 4: Mitigation Monitoring and Reporting Program.** This chapter identifies each significant impact and mitigation measure. The implementation responsibility, monitoring responsibility, and timing and performance standards are detailed for each specific mitigation measure.

- **Appendix A: BFFIP Project Environmental Review and Checklist.** This document details the actions to be taken for each individual project under the BFFIP. A flow chart for environmental review dictates the necessary documentation and review required.

2 RESPONSES TO COMMENTS

2.1 INTRODUCTION

This section contains the comments received during the public review period on the Draft Program EIR prepared for the BFFIP and the responses to those comments. Written and verbal comments on the Draft Program EIR were received from the organizations and private individuals identified in Table 2.1-1. No comments were received from State, regional, or local resource agencies during the public review period. A public meeting was held during the public review period at the District Main Office on April 10, 2019, to receive verbal comments. Four members of the public asked questions or made statements during the public meeting; the District has transcribed their comments and provided responses.

The comments are organized into three categories (organizations, individuals, public meeting) and are listed with the name of the commenter and the date their letter was received or verbal comment taken in Table 2.1-1. Each comment letter has been assigned a code as shown in the table. Each specific comment within a particular letter has been bracketed and assigned a number. For example, the third comment in letter "A3" is identified as "Comment A3-3." The corresponding response uses the same coding system. In this fashion, the reader will be able to identify the comment to which a response refers.

Table 2.1-1 Commenters on the Draft EIR and Corresponding Comment and Response Numbers

Commenter	Comment Code	Date of Comment
Organizations		
California Native Plant Society (CNPS), Carolyn Longstreth	A1	6/4/2019
Marin Group Sierra Club, Judy Schriebman	A2	6/11/2019
Marin Chapter of the California Native Plant Society, Carolyn and Paul DaSilva	A3	6/15/2019
Watershed Alliance of Marin, Laura Chariton	A4	6/17/2019
Friends of the Corte Madera Creek, Sandra Guldman	A5	6/19/2019
Marin Conservation League, Linda Novy	A6	6/19/2019
Marin Audubon Society, Barbara Salzman	A7	6/19/2019
Individuals		
Bill Rothman	B1	4/9/2019

Commenter	Comment Code	Date of Comment
Toni Shroyer	B2	4/10/2019
Martine Algier	B3	4/12/2019
Dora Howard	B4	5/4/2019
Mia Pritts	B5	5/13/2019
Roger Roberts	B6	5/29/2019
Ruth Todd	B7	6/11/2019
Georgia Gibbs	B8	6/12/2019
Christina Bertea	B9	6/13/2019
Lito Brindle	B10	6/18/2019
Larry Bragman	B11	6/19/2019
Larry Bragman	B12	6/19/2019
Aaron Gilliam	B13	6/19/2019
Public Meeting		
Marin Conservation League, Nona Dennis	C1	4/10/2019
Pesticide Free Zone, Ginger Souders-Mason	C2	4/10/2019
Larry Minikes	C3	4/10/2019
Marin Chapter of Native Plant Society, Eva Buxton	C4	4/10/2019

2.2 MASTER RESPONSES

2.2.1 Overview

This section contains master responses to address comments on topics that were raised multiple times. Master responses provide information in a comprehensive discussion that clarifies and elaborates upon, as necessary, the analysis in the Draft Program EIR. As appropriate, the responses to individual comments refer back to master responses.

2.2.2 Master Response 1: Definition and Location of New and Expanded Fuelbreaks

2.2.2.1 Comments

Several comments were made on how the Draft EIR defines the “project” under CEQA and on the adequacy of the project description as presented. Commenters stated that the project was not adequately defined under CEQA because the Draft EIR did not identify the specific locations where proposed fuelbreaks would be widened or created. A few commenters noted that Figure 2.7-1 on page 2-35 of the Draft EIR identifies the locations of the new and widened fuelbreaks but thought that the map did not provide enough detail due to its scale. One commenter noted that readers are referred to Figures 2.6-1 to 2.6-5 of the Draft EIR and Figures 3-12 to 3-16 of the BFFIP in the discussion of MA-21 (new fuelbreak construction); however, these figures only identify the categorization of existing fuelbreaks and do not show the new and expanded fuelbreaks. Some commenters appear to have confusion over the definition of a firebreak versus a fuelbreak when stating their concerns over impacts from fuelbreak creation.

The primary issue raised by most commenters concerned with the detailed mapping of new fuelbreaks and fuelbreak expansions, and the definition of the project, is how these new and expanded fuelbreaks affect rare and listed plant species.

2.2.2.2 Response

Definitions

As described in Chapter 2 Project Description, a fuelbreak is a swath or patch of land where dense vegetation has been thinned to reduce the fuels, increasing the success of suppressing a wildfire. A firebreak is a swath of land where vegetation has been entirely removed. Firebreaks are not proposed as part of the BFFIP. Medium-sized vegetation that acts as ladder fuels from the grass or forest floor to the crown of trees is minimized or eliminated within fuelbreaks. The type of fuelbreak proposed as part of the BFFIP can also be referred to as a “shaded fuelbreak” when conducted in forest or woodland habitats as the canopy remains intact. A study of shaded fuelbreaks generally[1] did not find nonnative plant cover to be statistically different in the treated fuelbreak area compared to adjacent wildland (Merriam, Keeley, & Beyers, 2007).

The Wide Area Fuel Reduction Zone (WAFRZ) is a natural area zone within which treatments to improve both fuels profile and ecosystem health through invasive species removal and forest management would occur. This zone is depicted on Figures 2.6-6 through 2.6-10 (pages 2-26 through 2-30 of the Draft EIR). Within this zone, larger areas of land, as opposed to linear swaths of land for fuelbreaks, are treated to thin vegetation and minimize fuel loads.

Existing Conditions and Size of Existing Fuelbreaks

The District has completed approximately 450 acres of formal, permanent fuelbreak system, which includes defensible spaces, and treated another 450 acres of WAFRZ since adoption of the 1995 VMP, for a total of 900 acres of fuel-load reduction. Existing permanent fuelbreaks are generally located along roadways or other infrastructure. The existing fuelbreaks are shown in Figure 2.3-1. It should be noted that fuelbreak widths are very narrow in relation to the overall size of the watershed. The fuelbreak widths in Figure 2.3-1, therefore, are not to scale. The widths of existing fuelbreaks for each type of fuelbreak are described in the March 2019 Draft BFFIP on page 3-20. Primary containment fuelbreaks are 100 to 200 feet wide, secondary

[1] One studied shaded fuelbreak had lower relative nonnative cover within the fuelbreak than the adjacent wildland.

containment fuelbreaks are 60 to 100 feet wide, and ingress–egress fuelbreaks are 15 feet wide, extending from either the edge of the road. As noted above, these fuelbreaks are not denuded of vegetation; rather, these fuelbreaks are areas in which vegetation has been thinned. WAFRZs can be up to 0.25 mile or more in width and are variable as the width depends upon the type of adjacent habitat and where it transitions.

Location of New and Widened Fuelbreaks
MA-21 describes the creation of new fuelbreaks. The District would construct, as a part of this plan, approximately 50 additional acres of fuelbreaks by the end of five years following plan adoption and an additional 67 acres over the lifetime of the plan for a total of 567 acres of fuelbreak, as stated on page 2-34 of the Draft EIR. Figure 2.7-1 depicts the locations of the 117 acres of proposed new and widened fuelbreaks. The proposed fuelbreaks would generally involve expansion of existing fuelbreaks, as opposed to creation of fuelbreaks in completely new areas and would be located along existing roadways and adjacent to other infrastructure.

The District received a comment stating that Figures 2.1-1 through 2.1-4 of the Draft EIR show only the zoning of the existing fuelbreaks. This statement is correct. The figures have been revised in the BFFIP and EIR to also show the new and expanded fuelbreaks and the associated infrastructure zoning that would be applied to the new and expanded fuelbreaks. The revisions are shown in Chapter 3 Revisions and Corrections of this Final EIR, which shows revisions to the analysis in the Draft EIR. The revised maps provide some additional detail as compared with the map in Figure 2.7-1; however, it should be noted that the width of the lines presented in the revised figures is still not to scale. Fuelbreak expansions are typically 100 feet wide or less. The width of the lines on Figure 2.7-1 suggests that the fuelbreaks will be wider than 100 feet. The scale of the lines as shown on the figure is a byproduct of the line drawing tools that have been used to prepare the figure. In fact, fuelbreaks are typically 100 feet wide or less.

Impacts to Rare Plants Found in New and Expanded Fuelbreaks
The level of detail presented in Figure 2.7-1 and the revised Figures 2.6-1 through 2.6-4 shows the general proximity of the new or expanded fuelbreaks adjacent to existing fuelbreaks. The impacts of the types of maintenance activities involved in fuelbreak creation and maintenance is analyzed in Section 3.3 Biological Resources of the Draft EIR. Rare plants that could occur by habitat type are identified in Section 3.3. Mitigation Measure (MM) Biology-2 (pages 3.3-124 through 3.3-125 of the Draft EIR) defines the measures to be implemented to protect rare plants. Prior to constructing new or expanded fuelbreaks, if rare plants are potentially present based on the presence of suitable habitat and surveys have not been performed within at least five years, then further surveys would be performed to identify rare plants. If found, MM Biology-2 identifies the actions to be taken to avoid or reduce impacts. The mitigation is not considered "deferred mitigation" because it does not involve the further discretion of staff to determine impacts. The measure spells out the standards and performance criteria that must be met if a rare plant species is found in an area of a new or expanded fuelbreak. Fuelbreaks would be created over the next several years and, therefore, it is more protective to prescribe surveys prior to fuelbreak construction and implement the identified avoidance and minimization measures of MM Biology-2 if sensitive species are found. Surveying the entire area is not

considered practical for two reasons. First, conditions may change over time, such that a given area would have to be resurveyed before a given fuelbreak is constructed or expanded. Second, new or expanded fuelbreaks may not be constructed on all the areas shown on Figure 2.7-1. For these reasons, if the entire area is surveyed now, much of this work may have to be repeated or may turn out to be unnecessary. The District would prefer to avoid the expense of performing unnecessary surveys, particularly where, as here, there is a commitment to perform pre-construction surveys at the time they are needed. The District notes that, particularly for biological resources, there are numerous examples of instances in which pre-construction surveys have been accepted as a component of appropriate mitigation.

Several other concerns over rare plants and MM Biology-2 were raised in the comments. See **Master Response 3: Special-Status Plants** for a discussion of rare plant impacts and mitigation.

2.2.3 Master Response 2: Ecosystem and Fuels Deferred Action Zone

2.2.3.1 Comments

A few commenters expressed concern about the impacts associated with deferring treatments in the infrastructure zone identified as the "Deferred Action Zone" (page 2-31 of the Draft EIR). The commenters suggested that deferring action should be a management action and the impacts of not maintaining the vegetation should be addressed and identified as a significant impact.

2.2.3.2 Response

District lands are broken up into zones. One of these zones is the Ecosystem and Fuels Deferred Action Zone, as shown in Figures 2.6-6 through 2.6-10 (pages 2-26 through 2-30 of the Draft EIR). Management actions prescribe activities designed to achieve the plan's overall goals. Each management action identifies strategies and locations (zones) where the strategies would be applied.

The Ecosystem and Fuels Deferred Action Zone is characterized by the dominance of large, persistent populations of perennial weeds, hard-to-access stands of diseased trees, lack of special-status species, and diminished ecosystem function. Vegetation management is a lower priority in this zone compared to areas where success can be more readily attained. The strategy for this zone is to defer large-scale action but contain weeds where strategically possible. Maintenance activities occur in this zone under existing conditions and would continue to occur following implementation of the BFFIP. No change in the management of this zone would occur compared to existing conditions as a result of plan implementation. Any environmental effects associated with the existing weeds and diseased trees are part of the baseline conditions for the EIR (CEQA Guidelines Section 15125). No new impacts would occur as a result of the BFFIP. In effect, existing environmental conditions in this zone would remain the same.

2.2.4 Master Response 3: Special-Status Plants

2.2.4.1 Comments

Several commenters requested clear maps comparing the locations of the new and expanded fuelbreaks and rare plants (as discussed under **Master Response 1: Definition and Location of New and Expanded Fuelbreaks**). Several commenters stated that, in their view, the mitigation measures for plant species are inadequate, deferred, or vague. They expressed concern that five-year intervals for plant surveys were not adequate and that such surveys should occur more frequently. Commenters also stated that the sensitivity rating of plant species, as defined in MM Biology-2, is not clearly identified. The commenters stated that "low sensitivity species" are not adequately identified and impacts to them are not properly mitigated. Commenters also expressed concern over the vagueness of the term "hand methods" with regard to vegetation management near special-status plant species.

Concern was raised about the potential for an influx of invasive species following fuelbreak creation, which would increase the number of weeds on the Watershed instead of reducing them. The commenters were concerned over the impact weeds would have on special-status plant species.

2.2.4.2 Response

Special-Status Plant Species Impacts

The project area encompasses approximately 21,600 acres. Therefore, only currently mapped locations of special-status plant species are shown in Figures 3.3-12 through 3.3-16 on pages 3.3-39 through 3.3-40 of the Draft EIR (and 2-9 to 2-14 on pages 2-19 through 2-24 of the March 2019 Draft BFFIP). Figure 3.3-21 Special-Status Plant Species Locations and BFFIP Zones, on page 3.3-92 of the Draft EIR, shows the locations of special-status plants in relation to the various zones, including the infrastructure zone, that generally encompass the new fuelbreaks as shown in revised Figures 2.6-1 through 2.6-4. It is acknowledged that the locations of special-status plants are not presented in detailed maps in the Draft EIR and individual rare plants are not shown on the maps. Known species occurrences are grouped together and shown by a single symbology. While individual species could be shown, the data is not comprehensive and may or may not reflect actual locations of rare plants at the time of fuelbreak construction (or any other activity). All rare plant species with habitat on District lands, however, were identified in the Draft EIR in Table 3.3-5 on pages 3.3-30 through 3.3-37. The table provides the requisite detail on the habitat in which the species can be found and the potential to occur on District lands. The types of impacts that could occur from BFFIP activities on rare plants, including fuelbreak creation and maintenance, are presented on pages 3.3-73 through 3.3-75, 3.3-91 through 3.3-92, and 3.3-97 through 3.3-98 of the Draft EIR. The impacts to any species of rare plant are related to manual and mechanical ground disturbance in the case of new or expanded fuelbreaks. Impacts include direct damage to or removal of individual plants or populations. The impacts would not differ based on species. The mitigation (discussed in more detail below) to reduce effects to rare plants focuses on surveying and identifying rare plants and habitat prior to work and on avoidance. The measure also identifies specific requirements depending on the special-status species of plant found and its life form if the species cannot be avoided. Revisions to bolster the mitigation measure have been included in the Final EIR, as noted below, and shown in Chapter 3 of this Final EIR.

Mitigation Measures
A five-year time limit for plant survey data is a reasonable approach. Approved wetland delineations, which include surveys for plant species and communities, are valid for five years, in accordance with the U.S. Army Corps of Engineers, Regulatory Guidance Letter No. 16-01, dated October 2016, which states that an approved Jurisdictional Delineation will remain valid for a period of five years (subject to certain limited exceptions explained in Regulatory Guidance Letter 05-02). If conditions were to change (such as a fire or listing of a new species), surveys could be completed more frequently by District staff. The District often follows up on known or previously mapped populations of rare plants on a more frequent basis.

The use of sensitivity ranking has been removed from MM Biology-2 per the commenter's concerns on implementation and determination of "low sensitivity." MM Biology-2 has been revised to address potential impacts on special-status plants with known rarity or declining populations and all other special-status plants with California Rare Plant Rank (CRPR) of 1B or 2. The revisions to the measure are shown in Chapter 3 of this Final EIR. Special-status plants with known rarity or declining populations on District lands would not be removed as part of any vegetation management activity. These plants would be flagged or demarcated and a buffer of 100 feet around the individual or population established. Hand methods would be used to carefully avoid the marked plant species. Hand methods would include hand pulling of vegetation or use of non-powered or powered hand tools so that the operator can be precise and avoid the plant. The mitigation measure has been revised to provide more specificity as to what is meant by hand methods, as shown in Chapter 3.

MM Biology-2 identifies mitigation for other special-status perennials and annuals with habitat on District land. These species are known rare or have declining populations, including CRPR 1B or 2. No net loss of these species can occur. The populations would be marked in the field and avoided if possible. If an individual or population must be removed, one or two options can be employed (subject to California Department of Fish and Wildlife [CDFW] approval) and monitoring conducted to ensure that no net loss of the population occurs. Options include 1) relocating/reseeding; or, 2) planting nursery-grown seedlings in appropriate habitat outside the work area or in the work area following completion of work. The BFFIP may have some impacts on other CRPR Rank 4 species (as identified in Table 3.5-5 on page 3.3-30 of the Draft EIR) not listed in part b of the revised MM Biology-2; however, impacts would not be considered significant because either these species are abundant and stable or the BFFIP would not have an impact on them as they are not documented on District lands.

2.2.5 Master Response 4: Wildlife

2.2.5.1 Comments

Several commenters stated that the Draft EIR did not address impacts to common wildlife species and their populations. Other commenters stated that the analysis focuses exclusively on the construction and active management phase of vegetation removal, and the commenters disagreed with the Draft EIR's conclusion that longer-term habitat alteration would be largely beneficial. The commenters stated that fuelbreaks create a barrier and exposure hazard for reptiles and small mammals and would increase invasive species on the Watershed.

Some commenters stated that the creation of new and expanded fuelbreaks would have substantial impacts on ground-nesting species and common wildlife by removing cover and habitat. Commenters also expressed concern that the Draft EIR did not address impacts to prey species of northern spotted owl following fuelbreak creation or maintenance, particularly woodrat nests. The commenters had specific concerns that mitigation did not protect woodrats because clearing around nests while avoiding nests would still expose the woodrats to significant predation and, potentially, population impacts.

2.2.5.2 Response

Significance Criteria under CEQA

The EIR was prepared in accordance with CEQA (PRC § 21000 *et seq.*) and the amended Guidelines for the Implementation of CEQA (CEQA Guidelines) (14 CCR § 15000 *et seq.*) to provide an assessment of the potentially significant environmental effects of the proposed plan. The resource topics and questions analyzed in the Program EIR are in accordance with Appendix G of the CEQA Guidelines. The CEQA Guidelines do not contain a question regarding substantial adverse effects on all common species. Several of the Appendix G questions pertain to certain common wildlife species, including nesting birds, migratory birds, and nursery sites or habitat corridors for native species, which are addressed in Section 3.3 Biological Resources of the Draft EIR.

Fuelbreak Impacts on Wildlife, including Ground Nesting Species, from Habitat Alteration

As discussed under **Master Response 1: Definition and Location of New and Expanded Fuelbreaks,** and in the Draft EIR, a fuelbreak is not a denuded area without trees or other vegetative cover that could create a barrier or an exposure hazard for small or slow-moving wildlife species. Section 2.8.2 of the Draft EIR describes the specific processes by which fuelbreaks would be created or maintained. For example, shrubs would be removed or thinned until spacing between individual shrubs or shrub islands is more than double the height of the canopy (e.g., for shrub canopies six feet in height, 12-foot gaps will be created). The forest canopy would be retained. Shaded fuelbreaks are vegetated, providing a degree of cover, foraging, and nesting habitat for species (as stated on page 3.3-122 of the Draft EIR) although density of vegetation is reduced. Predation of ground-dwelling birds, reptiles, or mammals would not increase substantially due to maintenance of cover vegetation on the ground and the abundant surrounding areas of habitat for these types of common species. New or expanded fuelbreaks represent a very small fraction of the watershed lands available for common wildlife (approximately 0.5 percent). While some increased predation is possible due to reduced density of vegetation in fuelbreaks, it would not substantially impact population sizes of common species since the habitat alteration is so limited compared with surrounding areas. Fuelbreaks proposed as part of the BFFIP would not create a major barrier for movement of wildlife or fragment habitat, and the analysis as presented in the Draft EIR is adequate.

Ground-nesting species tend to make their nests within protected areas where they can find clumps of grass or at the base of a shrub, where they are less visible. Ground nesting birds may be deterred from nesting in fuelbreak areas if the vegetation does not provide the protection that they typically need. As previously stated, new and expanded fuelbreaks would comprise only 0.5 percent of the overall plan area and, therefore, the loss of potential nesting areas should not have a substantial impact on populations of ground-nesting birds.

One of the goals of the BFFIP is to reduce invasive and weed species through various methods. Reducing invasive weeds to allow native species to diversify will benefit biodiversity. Common species, including small mammals and reptiles, will benefit from increased biodiversity.

Direct Impacts to Special-Status Species

Direct impacts on special-status animal species could occur from injury or death through direct contact with equipment used for vegetation removal. Noise from mechanical equipment and workers could impact animal species, as could smoke from prescribed burns, particularly during their breeding season. Hand-removal methods and planting generally would not have direct impacts on species given the limited noise and limited ground disturbance involved. Most species can move out of harm's way to prevent injury or death from activities performed by hand. Mitigation measures are identified to reduce impacts to less than significant levels for each species that could occur in the plan area, where appropriate. Some revisions were made to MM Biology-5: Roosting Bats to add specificity to the measure for impacts to roosting bats from prescribed burning. The revisions are shown in Chapter 3.

Impacts to Northern Spotted Owl from Habitat Loss and Loss of Prey Base

The impacts on northern spotted owl are analyzed in Section 3.3 Biological Resources of the Draft EIR. Only a small fraction of the overall Watershed would be impacted by any activities in a single year. Once management actions are complete, habitat health would improve over time. Some degree of habitat alteration would occur from removal of ladder fuels and invasive species. Several BMPs and mitigation measures would be implemented during work activities to minimize the risk of spreading invasive species and forest diseases.

Some vegetation management activities would involve removal of woody debris, which could result in destruction of woodrat nests, the main prey of the northern spotted owl. Mitigation measures require avoidance of woodrat stick nests to minimize impacts on northern spotted owl from diminished prey populations. The comment that avoiding woodrat nests may still expose woodrats if the area around the nests is cleared is noted. A study of dusky-footed woodrats in the redwood region of California did not find an association between abundances of woodrats and different intensities of forest thinning (Hamm & Diller, 2009). However, MM

Biology-14 has been revised to state under item 2 that woodrat stick nests and the areas around the nests would be avoided during vegetation management activities, as shown in Chapter 3. The locations of woodrat nests in relation to existing fuelbreaks have not been mapped. It should also be noted that woodrats prefer to build nests in dense chaparral and in areas near streams. These areas generally do not correspond to new fuelbreak areas. Population declines are not anticipated as few nests are expected to be impacted given the location of fuelbreaks and the limited acreage that would be impacted as compared with the plan area (0.5 percent of plan area is new or expanded fuelbreak).

2.2.6 Master Response 5: Grazing

2.2.6.1 Comments

Many commenters expressed an interest in and support for grazing as a management tool. Some commenters felt that grazing was not given enough attention or detail in the BFFIP and Draft EIR, as the activity was limited to MA-27, which is mostly for experimental studies. One commenter suggested grazing could replace several of the other methods of vegetation removal and requested that a side-by-side comparison of grazing and mechanical methods/prescribed burning be presented to show that grazing reduces greenhouse gas (GHG) emissions.

2.2.6.2 Response

The BFFIP allows for grazing as a tool that can be used for fuel load and invasive species management under MA-27. As part of MA-27, grazing may also occur to achieve the restoration and reintroduction objectives under MA-25 and MA-26. Grazing is fully analyzed throughout the Draft EIR in equal detail as mechanical and manual methods and prescribed burning. Should experimental trials show grazing to be successful, use of grazing can be expanded under adaptive management. Heavy grazing by domestic goats for four or five years during the growing season is reported to effectively control broom in New Zealand (Hosking, Smith, & Sheppard, 1996) and has been tried in Marin County. There are disadvantages to grazing as a means of reducing fuel loads and invasive species. Goats are not selective, and they also eat native species. Goats can be used in selective areas, but no alternative is identified or feasible that completely replaces equipment use or broadcast burning with grazing. Many management actions including MA-23 and MA-24, such as Douglas-fir thinning and sudden oak death (SOD) treatments, would not be achievable with grazing instead of use of mechanical equipment. Broadcast burning is a tool used to address not just fuel loading but also habitat enhancement. Under MA-27, the District could perform a study of grazing to understand its efficacy, the resources needed, and environmental impacts and to compare these parameters with other methods. The adaptive management approach of the plan could allow for greater use of grazing should data show that grazing is a better tool with fewer impacts. . Air quality and GHG impacts of all other methods, except prescribed burning, were less than significant as proposed.

2.2.7 Master Response 6: Limited Use of Herbicides Alternative

2.2.7.1 Comments

Support of Limited Use of Herbicides Alternative
Some commenters expressed support for the Limited Use of Herbicides Alternative and recommended adoption of this alternative over the proposed plan. Comments were made that community opposition to herbicides is not an adequate justification for rejection under CEQA. Those commenters stated that the community opposition is misplaced because it is based largely on widespread aerial spraying of glyphosate to control weeds of food crops, which is not the same as the "limited herbicide" alternative presented in the Draft EIR and as should be used in Integrated Pest Management (IPM). IPM with limited herbicide use is the standard approach of public land managers for weed control. Limited use of glyphosate is recommended by Cal-IPC despite its classification by the International Agency for Research on Cancer (IARC), a branch of the World Health Organization, as "probably carcinogenic to humans." Commenters brought up various studies and classifications debating glyphosate's toxicity, especially when considering the method of application proposed in the alternative. Supporters of the Limited Use of Herbicides Alternative also stated that herbicide use would result in removal of more invasive species with fewer impacts.

Opposition to the Limited Use of Herbicides Alternative
Other commenters suggested that the analysis of herbicide use under the Limited Use of Herbicides Alternative did not adequately describe the severity of impacts and that calling impacts to human health less than significant was incorrect. One commenter stated that "limited" was not defined and therefore the statements of effects were meaningless. The commenter also stated that the effects of endocrine disruptors are significant regardless of dose and that inert ingredients can be more toxic than active ingredients. The commenter identified studies that suggest glyphosate is a negative factor in SOD as it is taken up by oaks, weakening the tree and allowing opportunistic infections.

2.2.7.2 Response

Scientific Community
Under this alternative, the use of three conventional herbicides—Aquamaster® (53.8 percent glyphosate, isopropylamine salt), Garlon® 4 Ultra (60.5 percent triclopyr, butoxy ethyl ester), and Transline® (40.9 percent clopyrad, monoethanolamine salt) would be allowed in addition to all of the tools for weed control available under the proposed BFFIP.

Different agencies have come to varying conclusions about whether there are health risks associated with glyphosate use. As analyzed in Chapter 4 Alternatives to the Proposed Plan of the Draft EIR, and noted by some commenters, the IARC classified glyphosate as "probably carcinogenic to humans" (IARC, 2017). The community in Marin County has raised considerable concern resulting from this classification of glyphosate as "probably carcinogenic to humans." The District has not allowed herbicide use in the Watershed since 2005. Since release of the Draft Program EIR, the Agency for Toxic Substances and Disease Registry

(ATSDR) released the Draft Toxicological Profile for Glyphosate, which details scientific studies that show a link between glyphosate and animal and human health effects, including cancer (ATSDR, 2019). Conversely, the United States Environmental Protection Agency (USEPA) has maintained the classification of “Not Likely to be Carcinogenic to Humans” (USEPA, 2017). Several points identified in Comment Letter A3 (Marin CNPS) are valid regarding Cal-IPC’s continued recommendation for limited herbicide use of glyphosate for weed control despite the IARC’s classification. The Marin CNPS also noted that other agencies, such as the European Food Safety Authority, have recently reached different conclusions regarding toxicity from those of the IARC. The scientific community has not come to a consensus on the human and environmental health hazards of glyphosate.

While triclopyr and clopyralid have not been identified as potential carcinogens, they have not been extensively studied to conclusively rule out carcinogenicity. According to the National Pesticide Information Center (NPIC), more studies are needed to determine if triclopyr exposures could be linked to human cancer risks (as stated on page 4-29 of the Draft EIR). The USEPA had determined that they are unable to classify human carcinogenicity of triclopyr. There is only weak evidence for breast cancer in female rats and kidney tumors in male rats (National Pesticide Information Center, n.d.). Likewise, the USEPA has not evaluated the ability of clopyralid to cause cancer. No publicly available studies of the cancer-causing ability of clopyralid-containing products are known.

One commenter cited studies claiming that glyphosate intensifies and can hasten the spread of SOD. The comment is noted; however, other sources state that glyphosate is not known to spread or intensify SOD (UC Berkeley, 2019). Under the alternative, only small quantities would be used on target species and in controlled applications. Exposure of healthy oak and other SOD-susceptible trees to herbicides would be very minimal and, therefore, significant effects of glyphosate use to intensify SOD would not be expected.

Impacts

Animals, applicators, and the public could be exposed to varying concentrations of herbicide from spraying and other methods of controlled application as proposed as part of the Limited Use of Herbicides Alternative. Potential for exposure, however, would be very limited due to several protection measures that minimize the probability of the applicators, the public, and animals coming into unintentional contact with sprayed or applied herbicide. The limited potential for public exposure from the District’s use of herbicides provides reasonable assurance that the public would not experience acute or chronic effects, including endocrine effects. Given the uncertainty around the environmental fate of herbicides, however, the health effects on animals and humans pose a potentially greater effect than not using herbicides at all. The impacts are identified as less than significant due to the limited use and the many restrictions placed under the Limited Use of Herbicides Alternative that would greatly minimize exposure.

Herbicides have the potential to drift, leave residues, or be spilled, as identified by some of the commenters. Quantities that would be used under the Limited Use of Herbicides Alternative would be so small that these exposures would not have effects on water quality (and therefore would not have an impact humans and animals). Herbicide quantities proposed for use would be so limited that it would not be detectable in reservoirs (for example, 20 gallons of herbicide in the smallest reservoir of 114,047,850 gallons would be non-detectable). Herbicides were used on the Watershed from 1995 until 2005. MMWD has conducted at least annual monitoring of glyphosate since 1993 in its seven reservoirs, two plant influents, and its groundwater source (Sonoma County Water Agency). There have been no detections since testing began, and clopyralid was not detected, either (Grabow, 2012). The Meadow Club golf course, which is northeast of the plan area, utilizes herbicides. MMWD sampled the pond at the Meadow Club, to which all greens and fairways drain, and which drains to Alpine Lake, in late November 2010. Samples had non-detectable concentrations of herbicides (Grabow, 2012). Herbicides would only be used in compliance with numerous restrictions, as previously stated. The restrictions include limitations on quantity and frequency of use, timing, and proximity to water, which would minimize potential for contamination to less than significant. While impacts from herbicide use could be minimized, some impacts could still occur that would not occur under the proposed BFFIP.

The Limited Use of Herbicides Alternative would not minimize the potentially significant and unavoidable impacts on air quality and GHGs from prescribed burning. Impact conclusions would be the same, but the level of severity for several resource analyses would differ. The severity of impacts from erosion, noise, and traffic would be less, but the severity of impacts related to hazards would be greater than under the proposed plan.

Conclusion
The Draft EIR identifies that the use of herbicides has limited community acceptance. However, the alternative was not identified as the environmentally superior because it did not address the significant and unavoidable air quality impacts of the BFFIP from prescribed burning. Herbicides cannot replace prescribed burning, as herbicide use does not have the same ecological benefits as prescribed burning. Herbicide use, even limited use, could introduce new impacts related to health hazards, the extent of which is likely less than significant but not definitively known. The BFFIP is designed to reduce the impacts of invasive species and improve ecological health on the watershed, even without herbicides. The plan includes criteria and goals to be addressed to the District's Board annually. The adaptive management aspect of the program will allow for reassessment of methodologies and their effectiveness in meeting the District's targets.

Adoption of the BFFIP, as proposed, does not preclude future use of herbicides. Conditions on District lands or in specific locations may change in unforeseen ways. In the event more information becomes available from the scientific community regarding risks, and if a need for herbicide use on District lands arises, the District could propose to amend the BFFIP to include the limited use of herbicides. The District would perform further CEQA analysis to permit use of herbicides.

2.2.8 Master Response 7: Benefits of the BFFIP and Program EIR

2.2.8.1 Comments

Some commenters inquired about how the Program EIR would address and cover future activities under the BFFIP and whether additional CEQA review would be required and how the need for additional review would be determined.

2.2.8.2 Responses

In accordance with the CEQA Guidelines a "Program EIR is an EIR which may be prepared on a series of actions that can be characterized as one large project that are related in some way" (Section 15168(a)).

The California Supreme Court has described the use of program EIRs in the following manner:

> An advantage of using a program EIR is that it can "[a]llow the lead agency to consider broad policy alternatives and program wide mitigation measures at an early time when the agency has greater flexibility to deal with basic problems or cumulative impacts." ([CEQA Guidelines], § 15168, subd. (b)(4).) Accordingly, a program EIR is distinct from a project EIR, which is prepared for a specific project and must examine in detail site-specific considerations. (Id., § 15161.)
>
> Program EIR's are commonly used in conjunction with the process of tiering. [Citation.] Tiering is "the coverage of general matters in broader EIRs (such as on general plans or policy statements) with subsequent narrower EIRs … ." ([CEQA Guidelines], § 15385.) Tiering is proper "when it helps a public agency to focus upon the issues ripe for decision at each level of environmental review and in order to exclude duplicative analysis of environmental effects examined in previous environmental impact reports." (Pub. Resources Code, § 21093, subd. (a); see also [CEQA Guidelines], § 15385, subd. (b).)
>
> In addressing the appropriate amount of detail required at different stages in the tiering process, the CEQA Guidelines state that "[w]here a lead agency is using the tiering process in connection with an EIR for a large-scale planning approval, such as a general plan or component thereof … , the development of detailed, site-specific information may not be feasible but can be deferred, in many instances, until such time as the lead agency prepares a future environmental document in connection with a project of a more limited geographic scale, as long as deferral does not prevent adequate identification of significant effects of the planning approval at hand." ([CEQA Guidelines], § 15152, subd. (c).) " … "Tiering is properly used to defer analysis of environmental impacts and mitigation measures to later phases when the impacts or mitigation measures are not determined by the first-tier approval decision but are specific to the later phases" [Citation.].

> (*In re: Bay Delta Programmatic Environmental Impact Report Coordinated Proceedings* (2008) 43 Cal.4th 1143.)

The BFFIP appears to be well suited for the preparation of a program EIR. The BFFIP includes a series of actions that are related "geographically" and as "logical parts in the chain of a contemplated action" (CEQA Guidelines, § 15168(a)(1) and (2)).

As specific activities are proposed, the District will review them to determine whether the effects were adequately analyzed in the Program EIR (per Section 15168(c)). As part of this review, the District would identify mitigation measures and management actions adopted as part of the BFFIP and, if they are applicable to the proposed action, the District would incorporate them into its approval of the specific activities. If the effects of the activity were not examined in the Program EIR, then further environmental review would be performed. As part of the Mitigation Monitoring and Reporting Program that must be approved at the certification of the Final EIR (PRC Section 21081.6(a)(1)), a Project Environmental Checklist form has been prepared and is presented in Appendix A of this Final EIR. The form allows the District to document whether future activities are covered under the Program EIR, or if additional review is required. Completion of this form may occur concurrently with the preparation of each annual workplan, covering all activities anticipated for the upcoming year.

In the absence of an approved BFFIP and Program EIR, work could potentially continue under the 1995 Program EIR. The 1995 Program EIR, however, does not cover some of the actions included in the BFFIP, such as forestry actions to address SOD, increased weed treatment levels through manual and mechanical removal, and Douglas-fir thinning, among many others. In the District's view, these actions are part of an overarching program and, as such, the actions are well-suited to analysis in a program EIR.

Alternatively, the District could analyze each activity as a discrete proposal, subject to project-specific environmental review. The CEQA Guidelines state, "Where individual projects are . . . to be undertaken and where the total undertaking comprises a project with significant environmental effect, the Lead Agency shall prepare a single program EIR for the ultimate project. . . .Where one project is one of several similar projects of a public agency, but is not deemed a part of a larger undertaking or a larger project, the agency may prepare one EIR for all projects, or one for each project, but shall in either case comment upon the cumulative effect." (CEQA Guidelines, § 15165.) Such review could consist of a categorical exemption, an Initial Study/Negative Declaration, or an EIR.

Thus, CEQA does not require the District to prepare a program EIR. Rather, CEQA provides that an agency has discretion to prepare a program EIR, with project-specific review to follow, if the agency determines that this approach has merit. In this case, the District has concluded that approval of the BFFIP and Program EIR ensures compliance under CEQA regarding considerations of cumulative effects and the application of mitigation when performing vegetation management activities.

The BFFIP and Program EIR process provides additional benefits and protections. The checklist in Appendix A for the BFFIP requires documentation of program activities and their impacts. Additionally, if a permit from a resource agency is required, such as from the California Department of Fish and Wildlife, that agency can rely on the Program EIR to issue that permit.

2.3 RESPONSES TO ORGANIZATION COMMENTS

This section presents all comments received on the Draft EIR, including comments received during the public hearing on April 10, 2019, and responses to all comments received. Where a comment is addressed in a Master Response, that Master Response number is indicated.

From: Carolyn Longstreth <cklongstreth@gmail.com>
Sent: Tuesday, June 4, 2019 3:27 PM
To: Biodiversity, Fire and Fuels Integrated Plan EIR
Cc: Laura Lovett; Paul Da Silva
Subject: Fwd: Map of New/Widened Fire Roads on Mt Tam

Comment Letter A1

Hello Shawn-- Im with the California Native Plant Society and Laura Lovett forwarded your email to me. But I still have questions:

1. As I read the maps you cite (Starting at Plan at p 3-33), they seem to delineate zones for "optimized" "transitional" or "compromised" vegetation, but what we want to know is where are new fuelbreaks planned and where are fuelbreaks to be widened? Are we supposed to be able to figure it out from these zones? There may be a typo in your email because it would be unusual to list page numbers in descending order (3-33 to 3-7). Please clarify. A1-1

2. Similarly, you refer us to other maps (DEIR at 2-26- to 29) that show areas slated for Preservation, Restoration, Restoration with WAFB and deferred Action . Again, we are interested in the locations of new fuelbreaks and those that are planned for widening? Do these maps or any others show this?

Thanks for clarifying these points. --Carolyn

---------- Forwarded message ---------
From: **Laura Lovett** <laura@lauralovett.com>
Date: Mon, Jun 3, 2019 at 4:53 PM
Subject: Fwd: Map of New/Widened Fire Roads on Mt Tam
To: Carolyn Longstreth <cklongstreth@gmail.com>

Shaun's answer when asked for the specific maps of the proposed new fuel breaks and widening.

Begin forwarded message:

From: Shaun Horne <shorne@marinwater.org>
Subject: RE: Map of New/Widened Fire Roads on Mt Tam
Date: May 22, 2019 at 8:05:43 AM PDT
To: 'Laura Lovett' <laura@lauralovett.com>

Laura,

In the BFFIP Plan the maps appear on Pages 3-33 through 3-7. In the BFFIP EIR the maps appear on Pages 2-26 through 2-29. Documents can be downloaded from the link below.

Clarification, I presented on the wide area fuelbreaks, not widened fire roads. Very different.

https://www.marinwater.org/455/Biodiversity-Fire-and-Fuels-Integrated-P

1

Shaun Horne
Watershed Resources Manager

Marin Municipal Water District
220 Nellen Avenue
Corte Madera, CA 94925
t 415-945-1192
shorne@marinwater.org
Follow us on the Web, Twitter, Facebook and our Blog.

-----Original Message-----
From: Laura Lovett [mailto:laura@lauralovett.com]
Sent: Tuesday, May 21, 2019 2:26 PM
To: Shaun Horne
Subject: Map of New/Widened Fire Roads on Mt Tam

Hi Shaun,
When you spoke to the MCL Fire and the Environment Committee back in March, and shared MMWD's vegetation management plans for the Mt Tam Watershed, you showed a slide that highlighted the new areas you had identified for new and widened fire roads on the mountain, esp. toward to top of the ridges. Can you share that map with me? I've looked for it in MMWD's Vegetation Management Plan for Mt Tam without any success.

Many thanks,

Laura Lovett

--
Carolyn Longstreth
PO Box 657
10 Balmoral Way
Inverness CA 94937
415-669-7514

2

2.3.1 Letter A1: Carolyn Longstreth, California Native Plant Society

Response to Comment A1-1

The commenter is asking where new fuelbreaks are planned and widened in the plan. Refer to **Master Response 1: Definition and Location of New and Expanded Fuelbreaks.** New and expanded fuelbreak locations are shown in Figure 2.7-1 of the Draft EIR. Figures 2.6-1 through 2.6-4 have been revised to show the new and expanded fuelbreaks and their zoning in addition to the existing fuelbreaks and their zoning. The fuelbreaks are not shown to scale due to their narrow widths as compared with the overall watershed size, but the relative position of the new and expanded fuelbreaks as compared with the existing fuelbreaks is shown.

From: Judy Schriebman <judy@leapfrogproductions.com>
Sent: Monday, June 17, 2019 11:20 AM
To: Biodiversity, Fire and Fuels Integrated Plan EIR
Subject: Sierra Club Marin Group Comments on BFFIP DEIR
Attachments: SCMG MMWD BFFIP DEIR comment.pdf

Comment Letter A2

Dear Shaun:

Attached please find the Marin Group Sierra Club's comment letter on the DEIR for the BFFIP. A lot of good work has gone into the DEIR and BFFIP. We are very appreciative of the efforts MMWD is making to protect and preserve the watershed for all of us.

Please let me know that you received this.

Sincerely,

Judy Schriebman
Chair Marin Group Sierra Club

1

SIERRA CLUB
SAN FRANCISCO BAY
Serving Alameda, Contra Costa, Marin and San Francisco counties

MARIN GROUP
scmaringroup@gmail.com

June 11, 2019

Shaun Horne, Natural Resources Manager
Marin Municipal Water District
220 Nellen Avenue
Corte Madera, CA 94925
bffipeir@marinwater.org

RE: The MMWD Draft Program Environmental Impact Report for the Biodiversity, Fire, and Fuels Integrated Plan

Dear Mr. Horne and MMWD Board:

The Sierra Club Marin Group represents 6600 Marin County residents committed to environmental protection and ecological stewardship of our public lands. We support the mission statement and the three goals of the BFFIP in protecting our precious water supply, water quality and watershed lands.

A2-1

We are very pleased in general with the direction that MMWD has taken in preparing the BFFIP and with the programmatic DEIR prepared by Panorama Environmental, Inc. that will inform how that plan is to be carefully carried out.

However, as we all know, Climate Change has changed the game. Given these new realities, what used to be acceptable and workable mitigation efforts may now be either not useful or actually damaging to species of concern, to hydrologic and geologic integrity, water quality and cohesiveness of native plant communities. It is therefore vital to anticipate worse case scenarios when doing mitigation work. We cannot assume that what has worked in the past will work under current and ever-changing conditions. We support using the most current Adaptive Management Restoration science throughout the Project as more information becomes available. Climate Change impact modeling done in the 1990's began to predict a shift in the distribution of vegetation types as global warming progressed. We know that MMWD, in conjunction with Students and Teachers Restoring a Watershed (STRAW), used Point Blue Conservation Science's[1] diverse palette of plant species that might be the future on watershed land. This is commendable forward thinking and planning.

A2-2

Do the standards of mitigation go far enough? Mitigations can be a low bar. Instead we should also

---

[1] STRAW is under the auspices of Point Blue Conservation Science, https://www.pointblue.org

2530 San Pablo Ave., Suite I, Berkeley, CA 94702 Tel. (510) 848-0800
www.sierraclub.org/san-francisco-bay/marin

be focusing on what we can do to *enhance and rebuild* our forests, for example, consideration of the use of mycoremediation[2] and phytoremediation.[3] It appears that these bioremediation[4] techniques were not mentioned in the BFFIP document but should be explored for rebuilding pesticide treated and/or disturbed soils. Keyline water management techniques for relieving soil compaction, and planting on contour along with placing swales on contour for water retention, are also important slope techniques to be considered and utilized. A2-2

Carbon sequestration planning needs to be an active part in every action undertaken by the District. Vegetation management should include the information and practices from Marin Carbon Project to use on District lands *specifically* for carbon sequestration. The COMET Planner out of Colorado for Carbon Sequestration also lists what to do at your specific location to sequester carbon. As well as taking direction from the California Forest Carbon Plan, which concentrates on coniferous forests, MMWD should also explore and plan for options and practices that enhance carbon sequestration in other biomes already acting as carbon sequestration areas, such as coast chaparral, oak woodlands, meadows and grasslands, freshwater wetlands, and riparian zones. A2-3

The following are our specific comments on the DEIR, tied to the section page numbers:

- ES-2: We believe that grazing could and should be used much more widely as a tool for both vegetation removal to reduce fuel loads for fire safety as well as for the removal of invasive weeds. A2-4
- ES-7 Alternatives: While Alternatives are required under CEQA, we support the BFFIP as a Project in its "no herbicide use" approach to vegetation management. We do not support any of the alternatives presented. In the event of a true emergency in which a significant threat to the watershed has been identified and all non-herbicide options have been tried and failed, a decision to use herbicides must be approved by 80% of the MMWD Board. A2-5
- ES-11: Invasive Plants: Loss of habitat for nesting birds from Douglas Fir removal needs careful evaluation to reduce long-term impacts, including selective rather than wholesale A2-6

---

2 Mycoremediation is a form of bioremediation in which fungi-based technology is used to decontaminate the environment. https://en.wikipedia.org/wiki/Mycoremediation. See also *MYCOREMEDIATION: Fungal Bioremediation*, HARBHAJAN SINGH, 2006 Wiley & Sons, https://www.wiley.com/en-us/Mycoremediation%3A+Fungal+Bioremediation-p-9780471755012

3 Phytoremediation is the direct use of living green plants for in situ removal, degradation, or containment of contaminants in soils, sludges, sediments, surface water and groundwater. Contaminants such as metals, pesticides, solvents, explosives, and crude oil and its derivatives, have been mitigated in **phytoremediation** projects worldwide. Many plants such as mustard plants, alpine pennycress, hemp, and pigweed have proven to be successful at hyperaccumulating contaminants at toxic waste sites. https://en.wikipedia.org/wiki/Phytoremediation.

4 Bioremediation is a process used to treat contaminated media, including water, soil and subsurface material, by altering environmental conditions to stimulate growth of microorganisms and degrade the target pollutants. In many cases, bioremediation is less expensive and more sustainable than other remediation alternatives.

thinning. For removal of Broom and Barbed goatgrass, timing is also critical. A California multi-year study evaluated a late spring burn as best for barbed goatgrass suppression, followed by two to four years additional burning to eradicate the grass.[5] Broom seeds are scarified by fire to germinate so aggressive follow up years of additional burns are necessary here as well. Volatile oils in Scotch broom[6] can create hot flames so caution is needed when burning or it can bring fire to the tree canopy. A2-7

- ES-11 Impacts: An expert, skilled in the special status species and habitats, must be frequently on site, even after training, to oversee all work being done in the field. Stopping an error or correcting a problem while work is in progress can avoid serious habitat impact. We have seen significant damage done by unsupervised crews doing work in every Marin watershed. Training alone may not be enough to ensure quality. A2-8
- ES-14 Impacts: Impacts to soils from clearing vegetation can also be mitigated by leaving large wood on the ground. Logs are necessary habitat, shade soils to retain moisture, help keep sediment in place and seldom burn in fast moving fires. "Nurse logs" are well defined as vital for new tree growth and forest health. A2-9
- ES-15 Working on slopes and saturated soils: Take into account precipitation predictions *before* commencing work. In addition to the steepness of a slope, consider soil composition and saturation when using mechanical equipment and heavy machinery. GIS mapping is available that is comprehensive and should inform work timing, be site specific and time of year specific. Given the programmatic nature of this document, site-specific evaluations at the local level are necessary for any work plan. A2-10
- ES-16 GHG: Enhancement of carbon sequestration should be another action of this plan, in order to temper the effects of climate change. We refer to Warren Karlenzig's January 24, 2017 letter, included in Appendix A. We support his recommendations, as well as the work of the Marin Carbon Project and SPAWN's 10,000 Redwoods Project. Trees that are known carbon sinks should be planted first and immediately in any restoration project, utilizing the tree carbon calculator from the Center for Urban Forest Research. Protect, maintain and enhance existing biomes that are sequestering carbon naturally. A2-11
- ES-18: The potential for the spread of invasive non-plant organisms, such as New Zealand Mud Snails, from one watershed to another, also needs to be carefully avoided. Proper decontamination (e.g. cleaning boots, equipment, tires, tools) and California Department of Fish and Wildlife protocols[7] must be followed when moving between watersheds and creek areas. A2-12
- ES-18: "Non-filament based geotextiles" are still typically made of plastic. We need to use non-geosynthetic materials that are decomposable, non-toxic, non-polluting, non-plastic, non-hydrocarbon based to protect wildlife and the environment from non-biodegradable A2-13

[5] https://bioone.org/journals/invasive-plant-science-and-management/volume-8/issue-3/IPSM-D-14-00043.1/Burning-Controls-Barb-Goatgrass-iAegilops-triuncialis-i-in-California-Grasslands/10.1614/IPSM-D-14-00043.1.short

[6] https://www.invasive.org/gist/moredocs/cytsco01.pdf

[7] https://www.wildlife.ca.gov/Conservation/Invasives/Species/NZmudsnail

3

plastic. Straw wattles may not be used if wrapped in plastic netting, which has been known to trap and kill wildlife, as well as being a source of microplastics when it breaks down. A2-13

- ES-18, ES-27, Noise: Human activities such as hiking, biking, running, horseback riding, vehicle and construction work all create noise, and impact the background noise in wildlife's natural habitats. "Noise sensitive receptors" that are not included in the DEIR will also be wildlife, such as Northern Spotted Owl (NSO).[8] Researchers have already observed an ecological shift in California's Santa Cruz Mountains as coyotes in the Santa Cruz Mountains have started to alter their diets from daytime prey, such as squirrels and birds, to nocturnal prey, such as rats and rabbits. Dusk to dawn trail closures already are in effect in this area near Northern Spotted Owl nesting sites.[9] Expansion of this topic is needed to protect noise-sensitive species from impacts. A2-14

Mitigation Measures Tables

- ES-27, MM Biology-1, Training: Plan also needs to add in frequent jobsite oversight of work crews by trained biologist/botanist/soil geomorphologist/hydrologist, depending on project focus. Training alone may not be adequate to ensure the work is being done properly (which we have seen with other projects, e.g. where work continued during the rain causing sediment flows into the Coho Salmon habitat of Redwood Creek). A2-15
- ES-31, MM Biology-3, Prevent the Spread of Invasive Species: This list also needs to include New Zealand Mud Snail detection, prevention and decontamination when working near waters where this invasive species exists. A2-16
- ES-41, MM Biology-17, Protection of California Giant Salamander: Relocation of Pacific Giant Salamander is not recommended. The activity should be delayed or never done in order to protect this species. The Salamander lives underground most of the time and only comes up to breed in water and feed. A2-17
- ES-53, third bullet point: Shutting down equipment "when soils become saturated and unable to support the machines" is inadequate for soil protection. Soils become compacted and damaged when worked wet which can lead to erosion and sedimentation. Highly saturated soils should be evaluated and exception to cease work made without regard to a 48-hour dry period since different soil types, such as clay, ephemeral wetlands, and ephemeral stream headwaters retain more water and are therefore more prone to damage. Heavy equipment should not be used on wet soils. A2-18
- ES-54, final bullet point, comment/question for clarification: Text states, "...no substantial ground disturbing work...48 hours after a rain event, defined as 0.5 inch amount of rain within 48 hour or greater period." Should this be "...48 hours after a rain event defined as A2-19

---

8 https://www.fws.gov/arcata/es/birds/MM/documents/MAMU-NSO%20Harassment%20Guidance%20NW%20CA%202006Jul31.pdf

9 https://www.scientificamerican.com/article/humans-are-driving-other-mammals-to-become-more-nocturnal/
"We'll need to understand local dynamics to really understand how we should be changing management of wildlife populations or human activities...her team's paper offers one potential approach: creating landscapes with "temporal zoning," where people limit disturbances at certain hours."

4

0.5 inch (or greater) amount of rain within a 48 hour period"? A2-19

- ES-58, MM Hazards-2, Avoidance of the contaminated Mill Valley Air Force Station: Remediation of this site should also be seriously explored, as a threat of wildfire hitting this area and the unintended release of toxic legacy chemicals could present a major public safety issue. A2-20
- ES-74, Limited Use of Herbicides Alternative: Statements that "the *limited* use of herbicides in this alternative may result in a more effective plan" for various reasons and "proposed *limited* herbicides effects" and "none of the effects would be significant given the *limited* use of herbicides," while not defining "limited" in its usage here, makes these statements ambiguous and meaningless for purposes of evaluation for significant effects. Furthermore, effects ARE significant when dealing with toxic products that are endocrine disruptors, since they are not dose dependent, unlike other substances where the dose makes the poison.[10] Hence, the statement that none of the effects would be significant is false. Endocrine disrupting chemicals (EDCs) are known carcinogens and have long-term health effects.[11] Certain pesticides are residual in soils, harm soil biota, and are not broken down for years, despite manufacturers' claims.[12] In addition, "Inert" ingredients in pesticides can also be more toxic than the active ingredient[13] and are non-transparent, non-discoverable (due to Corporate Proprietary Trade Secrets) and not tested in combination with the listed Active Ingredient(s). Adjuvants and surfactants themselves can be more toxic than the active ingredients or can make the active ingredient more toxic than isolated tests show.[14] There are other long-term A2-21

---

10 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3138025/

11 http://www.eastbaypesticidealert.org/Clopyralid.htm Subchronic toxicity; Effects on Reproduction; Contamination of Water: "EPA described clopyralid as "very soluble" in water and "very mobile" in soil and concluded that it "has the potential to leach to ground water and/ or contaminate surface water… Despite this low level of use, the U.S. Geological Survey has found clopyralid in two of the twenty river basins it has sampled for pesticides… clopyralid was found in soil water samples at all depths and dates tested, up to 30 days after treatment and down to a depth of 1.8 meters (almost 6 feet)."

12 https://content.sierraclub.org/grassrootsnetwork/sites/content.sierraclub.org.activistnetwork/files/teams/documents/The_Unintended_Consequences_of_Using_Glyphosate_Jan-2016.pdf This document contains multiple references to toxicity studies on Glyphosate.

13 http://www.alt2tox.org/tox_profile-triclopyr.htm, Triclopyr Ecological Hazard Summary: "Butoxyethyl ester has a greater potential for surface-water runoff and waterway contamination than the triethylamine salt, due to its low soil adsorption capacity. Butoxyethyl ester and TCP (the most common breakdown product of triclopyr) **may pose significant risks to groundwater and surface water sources**.... Additionally, triclopyr has been shown to disrupt the normal growth and nutrient cycling properties of microorganisms, fungi, mosses and algae; all of which perform critical functions to maintain a healthy ecosystem."

14 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4862968/ "Agrochemical risk assessment that takes into account only pesticide active ingredients without the spray adjuvants commonly used in their application will miss important toxicity outcomes detrimental to non-target species, including humans. Lack of disclosure of adjuvant and formulation ingredients coupled with a lack of adequate analytical methods constrains the assessment of total chemical load on beneficial organisms and the environment. Adjuvants generally enhance the pesticidal efficacy and

5

dangers from the use of these products. Glyphosate, for example, is known to be a negative factor in Sudden Oak Death (SOD), as this herbicide is released from targeted vegetation into the soil where it can then be uptaken by oaks, weakening the tree and allowing other opportunistic infections, such as SOD, to come in.[15] Its action is that of a desiccant, leaving dead and dried up vegetation, which creates a more fire-prone landscape. A2-21

Section 2 Project Description comments

- Page 2-19, Section 2.6.2.5 Fuelbreaks Completed by Others: Any lease or easement agreement entered into by MMWD and a third party needs to hold the third party to the terms of the BFFIP for watershed protection. Fuelbreaks along the perimeter should also be managed via the BFFIP. A2-22

Section 3 Environmental Analysis comments

- Page 3.5-9, Soil Erosion, Causes of erosion on roads and trails: Not mentioned is the erosion when mountain bikers cut new trails or widen existing ones by riding off trail, "scoop" hillsides, ride illegally down hills, and gully trails by riding in rain on wet dirt roads. Certain patterns of erosion also cause pedestrians to widen trails to avoid the gullies and ruts by walking on the edges, further eroding the trail and widening it. County workers have also been damaging the inboard ditch by grading and scraping them during or prior to the rainy season, removing the vegetation that would slow erosion, leading to sediment runoff that could damage watershed and water quality. A2-23
- Page 3.5-9, Specific improvements to roads and trails: We suggest the District investigate Pacific Watershed Associates' *Updated Handbook for Forest, Ranch and Rural Roads*[16] for specific road building techniques that prevent erosion, e.g. by sloping the road and not putting in inboard ditches, where runoff is treated by vegetation on the outer side, and culverts are not necessary. Maintaining inboard ditches require heavy equipment scraping of the vertical slope that causes excess erosion and sediment during the rainy season. A2-24
- Page 3.7-6 Hazardous Materials and Fire Hazards, subsection Topography: Riparian areas are also cooler, like north-facing slopes and less prone to fires. The US Forest Service[17] states, "Riparian areas (areas on or near the bank of a river, or other body of water) are transition areas between terrestrial and aquatic ecosystems and are typically highly productive areas. They also have an important function through *buffering the effects of fires* and other watershed disturbances on aquatic ecosystems. Due to higher levels of moisture, riparian areas next to streams and rivers can disrupt the spread of fire within a landscape." As such they can provide safe haven for firefighters as well as wildlife, as they act to cool and slow A2-25

---

inadvertently the non-target effects of the active ingredient."

[15] Source of this information is Dr. Don Huber, Emeritus Professor of Plant Pathology, Purdue University

[16] https://www.pacificwatershed.com/sites/default/files/RoadsEnglishBOOKapril2015b.pdf

[17] https://www.fs.fed.us/psw/topics/fire_science/ecosystems/riparian.shtml

6

the fire. Protection of the vegetation in riparian areas is important for fire safety and this should be added into the EIR here. A2-25

Appendices

- Appendix G, Cultural Resources Memo, Page 1 shows the usage of herbicides in contradiction to the BFFIP. "Vegetation management will also include weed control and utilize manual and mechanical techniques, prescribed burning, and herbicides for existing fuelbreak maintenance and defensible spaces." And from Page 29, "The proposed management and control actions including prescribed burning, removal via equipment and herbicides, among others all possess the potential to change the known cultural resources." A2-26 It is also of major concern "that nearly all of the resources have not been formally evaluated and/or field reviewed to determine their current location and status." Again, given the programmatic nature of this EIR, a site-specific evaluation should be made before a project is undertaken. A2-27

Thank you for considering our comments.

Sincerely,

Judy Schriebman, Chair Marin Group Sierra Club

7

2.3.2 Letter A2: Judy Schriebman, Marin Group Sierra Club

Response to Comment A2-1

The commenter notes that the Sierra Club is pleased with the direction MMWD has taken regarding the programmatic DEIR. The comment is noted.

Response to Comment A2-2

The commenter suggests the use of mycoremediation and phytoremediation as mitigation to enhance and rebuild forests to combat climate change by rebuilding pesticide treated and/or disturbed soils. The comment regarding climate change and its influence on the natural environment is noted. The BFFIP was developed in part to address the threats facing District lands. Climate change is one of the four key threats, as identified in Section 2.4.1 of the Draft EIR. The shift to hotter, drier climates and relationship to increased fire severity and frequency as well as effects on wildlife is acknowledged and accounted for in the BFFIP.

The commenter questions if the mitigation in the Draft EIR "goes far enough." In accordance with CEQA caselaw, mitigation measures must have an essential nexus and be generally proportional to the identified adverse impact (14 CCR, § 15126.4(a)(4)(A), (B), citing Nollan v. California Coastal Com'n (1987) 483 U.S. 825, 837; Dolan v. City of Tigard (1994) 512 U.S. 374, 391; Ehrlich v. City of Culver City (1996) 12 Cal.4th 854, 866-877). The commenter mentions that the mitigation should include bioremediation[2] methods such as mycoremediation[3] and phytoremediation[4] to treat contaminated soils and water. Implementation of the plan, however, would not result in soil or water contamination that would require such mitigation. These techniques have not been included in the plan as the plan is a vegetation management plan and remediation activities are not a part of its purview. An EIR is not required to mitigate for effects that would not result from the action proposed.

Mitigation measures were prepared to address each identified impact of the BFFIP's implementation using the best available science. Refer to Section 3.3 Biological Resources, Section 3.5 Geology and Soils, Section 3.6 Greenhouse Gases, and Section 3.8 Hydrology and Water Quality of the Draft EIR for analyses of the resources mentioned.

---

[2] Bioremediation is the use of either naturally occurring or deliberately introduced microorganisms or other forms of life for decontamination

[3] Mycoremediation is a form of bioremediation in which fungi-based technology is used to decontaminate the environment

[4] Phytoremediation is a form of bioremediation that uses various types of plants to decontaminate the environment

Response to Comment A2-3

The commenter suggests that carbon sequestration need to be an active part in actions undertaken by the District, including vegetation management. The commenter notes that MMWD should explore options that enhance carbon sequestration in other biomes already acting as carbon sequestration areas. The comment is noted as is the suggestion that the BFFIP should include actions to enhance carbon sequestration in other biomes. Refer to Section 3.6 Greenhouse Gases of the Draft EIR for an analysis regarding whether the BFFIP could substantially decrease the overall ability of District lands in the plan area to sequester carbon. Chipping, mulching, and burning would result in a temporary drop in carbon in live vegetation. In the long-term, the carbon losses would be offset by increased growth of existing trees and improved forest health, as identified on pages 3.6-11 through 3.6-17 of the Draft EIR. The impact on carbon sequestration within District lands from implementation of the BFFIP, as proposed, would be less than significant.

The commenter mentions that direction should be taken from and information incorporated from the Marin Carbon Project, the COMET-Planner, and the California Forest Carbon Plan to explore and plan for options and practices that enhance carbon sequestration in other biomes already acting as carbon sequestration areas, such as coast chaparral, oak woodlands, meadows and grasslands, freshwater wetlands, and riparian zones. The Marin Carbon Project is a consortium of agricultural institutions who seek to enhance carbon sequestration in rangeland, agricultural, and forest soils. The COMET-Planner is a tool to predict the carbon benefits from implementing recommended National Resource Conservation Service practices on farms and ranches. These references and tools are noted; however, the plan is focused on vegetation management in open space and forest land and not specifically on actions to increase carbon sequestration in agricultural practice. The plan does not preclude the use of practices to enhance carbon sequestration as part of vegetation management activities, where it is relevant to vegetation removal and mulching or replanting.

The California Forest Carbon Plan is discussed on page 3.6-11 of the Draft EIR. The Forest Carbon Plan identifies acceptable forestry actions that may have some short-term reductions in carbon sequestration for long-term benefits of reduced catastrophic wildfire. The BFFIP is consistent with the 2018 Forest Carbon Plan as stated in Section 3.6 of the Draft EIR.

Response to Comment A2-4

The comment notes that grazing should be used more widely as a tool for vegetation removal and removal of invasive weeds. Refer to **Master Response 5: Grazing** for more information about grazing as a tool for vegetation management under the BFFIP. The plan allows for grazing, but more extensive use may not be appropriate given the types and extent of weeds present.

Response to Comment A2-5

The commenter supports the BFFIP as a project in its “no herbicide use” approach to vegetation management. The comment is noted regarding the commenter’s support of the BFFIP for its “no herbicide use” approach and the Board requirements for approval of any future decisions to use herbicides. Refer to **Master Response 6: Limited Use of Herbicides Alternative** for a discussion of the herbicide alternative.

Response to Comment A2-6

The commenter explains that fir removal needs to be evaluated to reduce long-term impacts, including selective rather than wholesale thinning. Douglas-fir thinning is described on pages 6-9 and 6-10 of the March 2019 Draft BFFIP. The focus would be on selective removal of mid-canopy Douglas-fir and in areas where it is encroaching on grassland and oak woodland habitat. The BFFIP states,

> Priority is given to grasslands and oak woodlands where Douglas-firs are small, restricted to the margins, and/or are present in small numbers… The vast majority of Douglas-firs removed will be less than 12 inches DBH; limbs will be removed and piled for burning and trunks left in contact with the ground to decompose. Some larger Douglas-firs (up to 24 inches), or conifers that will damage oaks if felled, may be girdled and left as habitat trees.

Impacts associated with habitat alteration (including for avian species) are discussed on page 3.3-77 of the Draft EIR. The analysis states that

> …nesting birds, including special-status avian species, would have abundant areas to nest, even given management actions that may result in removal of dead trees and thick understory. Only a small fraction of the overall Watershed would be impacted by any activities in a year. Once management actions are complete, forest health would improve over time. Healthy forests would provide more native species and diversity and a more diverse prey-base, supporting the overall ecosystem health.

Nesting birds, including special-status avian species, would have abundant areas to nest, even given management actions that may result in removal of dead trees and thick understory, such as Douglas-fir thinning.

Response to Comment A2-7

The commenter notes that the timing of Broom and Barbed goatgrass removal needs to be considered. The comment is noted regarding timing of barbed goatgrass removal, risks of burning Scotch broom, and broom germination after prescribed burning. The District would conduct invasive species management using industry practices and knowledge from decades of management on District lands. Safety precautions implemented during prescribed burning are described on pages 3.7-25 to 3.7-26 of the Draft EIR. Vegetation would be pre-treated to reduce the potential for uncontrolled spread of fire, such as into the canopy.

Response to Comment A2-8

The commenter notes that an expert in special-status species and habitats must be on site frequently to oversee work being done. The recommendation for having an on-site expert, skilled in special-status species and habitats, is noted. The mitigation does not require the presence of a biologist with every work crew, as it is not practical. Several measures beyond just training would serve to protect sensitive biological resources and habitats when work is being performed. These protections are integrated into the planning phase of the work. The measures primarily require surveys by qualified biologists and avoidance of any resources or species found. These measures would reduce the potential for inadvertent damage by workers to sensitive species. For example, MM Biology-2 requires surveys prior to work and flagging and avoidance of special-status plant species. MM Biology-5 requires focused tree and habitat assessments to minimize impacts to roosting bats and a specific roosting bat protection plan. MM Biology-6 requires surveys for badger dens prior to burning or use of heavy equipment in denning habitat and avoidance or passive relocation by a qualified biologist. MM Biology-7 requires nesting bird surveys prior to certain types of work that could disturb nesting activity and implementation of protective buffers by a biologist if active nests are found. MM Biology-8 includes several measures to protect northern spotted owl, including surveys, establishment of buffers, and avoidance. MM Biology-9, MM Biology-10, and MM Biology-12 require surveys for western pond turtles, California red-legged frogs, and foothill yellow-legged frogs and moving or avoiding individuals if found. MM Biology-15 and MM Biology-16 require surveys for and protection through flagging and avoidance of wetlands and native grasslands.

Mitigation to minimize or avoid impacts is required. If the District finds risks of non-compliance with mitigation are valid, the District can, as part of their implementation strategy, have a biologist on site to ensure that mitigation is being implemented.

Response to Comment A2-9

The commenter notes that impacts to soil from clearing vegetation can also be mitigated by leaving large wood on the ground. The recommendation to leave large logs in place to reduce impacts to soils is noted. Refer to Section 3.5 Geology and Soils of the Draft EIR for an analysis of erosion and loss of topsoil. MM Geology-1 requires short- and long-term erosion control measures for areas at risk of erosion and loss of topsoil, including use of logs (page 3.5-39 of the Draft EIR). Impacts to soils would be less than significant with implementation of the proposed mitigation.

Response to Comment A2-10

The commenter notes that precipitation predictions must be taken into account before commencing work. The commenter also states that soil composition and slope steepness should be considered prior to using heavy equipment. MM Geology-1 requires a site inspection prior to conducting any management action that may result in erosion or slope instability, including consideration of slope and soil compaction, and many other factors like ground cover. The slope and existing conditions of a work area would be considered to determine the erosion control measures and restrictions that would be appropriate. Many of these restrictions apply to specific times of year, such as rainy season. Revisions have been made to MM Geology-1 to clarify that prior to conducting ground disturbing work, the weather forecast would be consulted to determine if rain is predicted. Revisions to the mitigation are shown in Chapter 3.

Response to Comment A2-11

The commenter states that enhancement of carbon sequestration by planting trees should be another action of this plan to combat climate change. The comment regarding enhancement of carbon sequestration is noted. Refer to Responses to Comments A2-2 and A2-3.

Response to Comment A2-12

The commenter notes that the potential of invasive species to spread between watersheds should be avoided by implementing proper decontamination techniques. New Zealand mud snails are not currently known to occur on District lands; however, the sighting referred to by the commenter was in San Anselmo Creek in the Town of Fairfax, in close proximity to Watershed lands. The vegetation management activities proposed as part of the BFFIP would generally avoid riparian corridors, and no in-stream or reservoir work is proposed. The potential for encountering and spreading invasive aquatic species during implementation of the BFFIP is very low and considered less than significant and, therefore, does not merit mitigation under CEQA.

Response to Comment A2-13

The commenter states that non-geosynthetic materials should be used to protect wildlife to avoid polluting the area with plastic that is non-biodegradable. MM Geology-1, as presented in the Draft EIR, requires erosion control measures and non-filament-based geotextiles to be approved prior to use. Approved control measures would not be permitted to cause harm to wildlife species or other impacts. MM Geology-1 has been revised, as shown in Chapter 3, to indicate that the non-filamentous-based geotextiles should also be biodegradable and biobased.

Response to Comment A2-14

The commenter states that noise-sensitive receptors, such as the Northern Spotted Owl, should be protected from construction noise or recreationalists as a result of the plan. Northern spotted owl and other special-status wildlife are not noise-sensitive receptors for the purposes of the analysis presented in Section 3.9 Noise of the Draft EIR; however, noise impacts on these species was thoroughly addressed in the Draft EIR in Section 3.3 Biological Resources.

Any effects associated with recreationalists or other ongoing activities is a part of the baseline conditions (CEQA Guidelines Section 15125). Section 3.3 Biological Resources of the Draft EIR analyzes the effect of noise from implementation of the BFFIP on special-status wildlife, including northern spotted owl. Noise from vegetation management activities could directly impact northern spotted owl. Table 3.3-8 presents analyses of direct impacts from each vegetation management tool and technique. Noise impacts could occur from use of heavy equipment and vehicles. MM Biology-1 (Worker Training) requires a training program that describes special-status species and how to avoid harming the species for all staff, contractors, or volunteers who would perform vegetation-management work. MM Biology-8 (Northern Spotted Owl Avoidance During Nesting Season) requires avoidance of noise-generating activities within 0.25 mile of an active nest until young have fledged or to determine a minimum buffer needed to avoid impacts on northern spotted owls from noise generation. Manual methods would not be allowed within 131 feet of line-of-site to a nesting pair. In accordance with the analysis, MM Biology-1 and MM Biology-8 would reduce direct impacts on northern spotted owl to less than significant.

Response to Comment A2-15

The commenter states that the plan needs to add in frequent jobsite oversight of work crews by trained biological specialists, depending on the project's focus. The recommendation to add frequent jobsite oversight by a biologist is noted. Refer to Response to Comment A2-8. Several mitigation measures in Section 3.3 Biological Resources, Section 3.5 Geology and Soils, and Section 3.7 Hydrology and Water Quality of the Draft EIR include requirements for pre-activity surveys or data review in addition to worker training, with specific requirements and restrictions dependent upon the survey results. The District can, as part of its implementation strategy, have a biologist or other technical specialist on site to ensure that mitigation is being implemented in areas when risks of non-compliance are deemed to be greater than typical.

Response to Comment A2-16

The commenter states that the plan should prevent the spread of the invasive New Zealand Mud Snail by implementing decontaminating techniques when working in waters near where the species exists. Refer to Response to Comment A2-12 regarding the New Zealand mud snail. Implementation of the BFFIP is not likely to result in the spread of New Zealand mud snails since it would not include instream activities.

Response to Comment A2-17

The commenter states that the relocation of the giant salamander should be avoided to protect the species. California giant salamanders are typically nocturnal but are sometimes active during daytime in wet weather. They are typically found close to permanent bodies of water. Encountering a California giant salamander would be unlikely during implementation of the BFFIP for these reasons. Should a California giant salamander be encountered during work, MM Biology-17 allows for a qualified biologist to safely and legally guide the individual California giant salamander out of harm's way or to avoid the area. The mitigation serves to preserve and prevent injury to any individuals encountered. Impacts to California giant salamanders would be less than significant with mitigation.

Response to Comment A2-18

The commenter notes that heavy equipment should not be used on saturated soils and that a 48-hour dry period should occur prior to commencement of work. Refer to Response to Comment A2-10. The third bullet under MM Geology-1 prevents heavy equipment from operating on saturated soils if it has rained within 48 hours, as the commenter states. MM Biology-15 requires heavy equipment used in wetland areas to be designed to operate within wet or saturated soils. Both mitigation measures would ensure that heavy equipment does not cause rutting, erosion, or compaction of soils, including in sensitive wetland soils.

Response to Comment A2-19

The commenter questions if ES-54 (final bullet point) should read "…no substantial ground disturbing work…48 hours after a rain event defined as 0.5 (or greater) amount of rain within a 48-hour period." Refer to Response to Comment A2-10. The measure has been revised for clarity to include that prior to conducting ground disturbing work, the weather forecast would be consulted to determine if rain is predicted, as shown in Chapter 3 of this Final EIR.

Response to Comment A2-20

The commenter states that remediation of the contaminated Mill Valley Air Force Station should be avoided due to wildfire threats. The recommendation to remediate the Mill Valley Air Force Station is noted. Refer to Response to Comment A2-2 that details the requirement for mitigation measures to have an essential nexus and be proportional to impacts. Implementation of the BFFIP would not cause wildfires but is rather intended to reduce wildfire risk. MM Hazards-2 requires avoidance of areas within the Mill Valley Air Force Station, which would ensure that any impacts that could occur from disturbing contamination during vegetation management activities is entirely avoided. Remediation of the Mill Valley Air Force Station is beyond the scope of the BFFIP and EIR.

Response to Comment A2-21

The commenter states that the use of "limited" should be more defined as it relates to use of herbicides. The commenter also notes that there are long-term dangers that should not be considered less than significant on human health when using toxic pesticides that are endocrine disruptors. The inclusion of the word "limited" in the alternative's moniker is defined in substantially more detail in Section 4.5.4, on pages 4-22 through 4-26 of the Draft EIR. This section of the Draft EIR identifies the three conventional herbicides that would be used under the alternative, Aquamaster® (53.8 percent glyphosate, isopropylamine salt), Garlon® 4 Ultra (60.5 percent triclopyr, butoxy ethyl ester), and Transline® (40.9 percent clopyrad, monoethanolamine salt) and the conditions under which they could be used.

Refer to **Master Response 6: Limited Use of Herbicides Alternative** for a discussion of why the impacts of herbicide use are considered less than significant given the numerous precautions and requirements for application. The methods of application would minimize or avoid public exposure to herbicides, which would avoid or reduce impacts. Herbicide impacts on SOD and herbicide drying that can increase fire risks are also addressed in **Master Response 6: Limited Use of Herbicides Alternative.**

Response to Comment A2-22

The commenter states that any leases entered into by MMWD and a third party needs to hold the third party to the terms of the BFFIP for watershed protection. The comment regarding lease agreements with other parties is noted. Agencies managing land adjacent to District lands or within District lands have developed and implement their own management plans. Different parcels of land have unique management issues and challenges. While the District coordinates with the surrounding jurisdictions and the intent is to develop mutually beneficial management actions for shared boundaries, the District cannot impose the BFFIP on fuelbreaks on the perimeter of the District but not owned by the District (as implied by the comment). Easements within District lands are generally subject to the requirements imposed on all District lands, including for vegetation management. Entities such as Pacific Gas and Electric Company (PG&E), however, have other vegetation requirements that must be met, as imposed by the

California Public Utilities Commission, to address the threats unique to their infrastructure. The BFFIP only covers and dictates the activities to be conducted by the District.

Response to Comment A2-23

The commenter states that erosion caused by mountain bikers cutting new trails and county workers performing grading activities is not addressed. The erosion impacts associated with implementation of the BFFIP are analyzed in Section 3.5 Geology and Soils of the Draft EIR. The list presented under Section 3.5.2.4 of the Draft EIR is not a complete list, but rather identifies the major contributors according to the Mount Tamalpais Roads and Trail Management Plan. The additional contributors to erosion on the Watershed identified by the commenter are noted but adding these contributors would not change the impact analysis as presented in the Draft EIR.

Response to Comment A2-24

The commenter states that specific road building techniques that prevent erosion as covered in the Updated Handbook for Forest, Ranch and Rural Roads published by Pacific Watershed Associates should be investigated. The recommendation to investigate Pacific Watershed Associate's Updated Handbook of Forest, Ranch and Rural Roads is noted. No new roads and trails are proposed as part of the BFFIP. Former logging skid roads could be cleared temporarily to access sites; however, permanent or significant road work would not occur. No grading or scraping would occur, and no material or base would be added. The recommendations of the commenter are not relevant to the BFFIP.

Response to Comment A2-25

The commenter notes that protection of vegetation in riparian areas is important for fire safety and should be added in the DEIR discussion. The recommendation to protect the vegetation in riparian areas is noted. The information provided in the setting of Section 3.7 Hazardous Materials and Fire Hazards of the Draft EIR pertains to general characteristics that influence flammability. The section acknowledges that fuel with low moisture and high quantities of dead biomass are more flammable (page 3.7-5 of the Draft EIR), which would indicate that moist and lush environments, such as those found in riparian areas, would be less flammable.

Impact Biology-2 in Section 3.3 Biological Resources of the Draft EIR provides a clear understanding of the types of activities that could not and would not occur in riparian areas. Vegetation removal in riparian habitat would generally consist of invasive species removal. Broadcast burns could occur within riparian habitats, but MM Geology-1 prohibits broadcast burning within a 50-foot buffer around perennial and intermittent streams when the broadcast burn is proposed on a slope greater than 30 percent and upslope of the stream (and, therefore, associated riparian habitat) so that impacts would be avoided. Riparian corridors would not be significantly impacted by implementation of the BFFIP.

Response to Comment A2-26

The commenter notes that Page 1 of the Cultural Resources Memo in Appendix G shows the usage of herbicides contradicts the BFFIP. The language in Appendix G Cultural Resources

Memo erroneously mentioned herbicides as part of the proposed BFFIP. The language has been revised to remove herbicides and reflect the language of the BFFIP, as shown in Chapter 3.

Response to Comment A2-27

The commenter notes that a site-specific evaluation should be made to address the potential to encounter cultural resources. Impacts on cultural resources from implementation of the BFFIP are analyzed in Section 3.4 Cultural and Tribal Cultural Resources of the Draft EIR. MM Cultural-2 requires the District's program manager to review confidential Geographic Information Systems (GIS) data prior to conducting work to determine if the area has been surveyed previously and whether any resources were found. If the GIS data shows that the areas where soil disturbance below the surface through use of heavy equipment or burning is proposed have not been previously surveyed, a pre-activity cultural resources survey would be conducted by a qualified archaeologist or cultural resources specialist in accordance with industry standards prior to performing work. In the event vegetation is too dense, making a pre-activity survey challenging or impossible, the training conducted under MM Cultural-1, would be sufficient to permit work to be conducted using only manual techniques accessed on foot. This measure is consistent with the commenter's recommendation.

Comment Letter A3

Marin Chapter
California Native Plant Society

June 15, 2019

Mr. Shaun Horne, Natural Resources Manager
Marin Municipal Water District
220 Nellen Avenue
Corte Madera, CA 94925

Email:
Shaun Horne, Natural Resources Manager
bffipeir@marinwater.org (maximum file size is 10 megabytes)

Dear Mr.Horne:

The Marin Chapter of the California Native Plant Society ("Marin CNPS") submits the following comments on the Draft Environmental Impact Report (DEIR) for the MMWD's Biodiversity, Fire and Fuels Integrated Plan (BFFIP). CNPS is an organization of nearly 10,000 members statewide dedicated to conserving native plants and their natural habitats and to increasing the understanding, appreciation, and horticultural use of native plants. Marin CNPS has approximately 350 members. A3-1

Briefly stated, Marin CNPS maintains that (1) the DEIR's description of the project is inadequate in that it fails to identify where fuelbreak construction or widening projects are to occur and how such projects relate to the locations of special status plant species and communities; (2) the mitigation measures for rare plants are vague and inadequate, particularly where such species lie within the path of a new or widened fuelbreak; and (3) the DEIR improperly categorizes special status plant species as low or high sensitivity. Finally, (4) we support the use of broadcast burning as a vegetation management tool even as we urge the District to abandon its misguided policy against herbicide use. A3-2

1. The DEIR fails to adequately describe the project.

The California Environmental Quality Act (CEQA) requires an EIR to "identify and focus on the significant effects of the proposed project on the environment. …. Direct and indirect significant effects of the project shall be *clearly identified and described*, given due consideration to both the short-term and long-term effects. The discussion should include *relevant specifics* of the *area, the resources involved, physical changes,* A3-3 *alterations to ecological systems and changes induced in population distribution, population concentration….*". CEQA Guidelines Section 15126.2 [emphasis added].

The DEIR fails to disclose where specific fuelbreak construction or widening projects are to be undertaken, nor the manner in which such sites relate to the locations of specific rare plants.

Management Action 21 (Construct the remainder of the fuelbreak system) states that a total area of 117 acres area will be affected, with first 50 acres to be completed within first 5 years of plan adoption. BFFIP ES-8; DEIR 2-36; 2-.6-1 to 2.6-1.65. This plainly indicates that MMWD knows how many of these projects will be done and where, yet this critical information is omitted from the Plan and from the DEIR. The reader is referred to Figures 3-12 to 3-16 for details on the fuelbreak system but these maps merely show how the existing fuelbreaks are categorized as "optimized, "transitional" or "compromised." These categories dictate the extent of management actions planned, but no map depicts the locations of all *new* fuelbreaks or those to be *widened*, which is, of course, of particular concern to CNPS. A3-3

The DEIR does include a map of planned fuelbreak activity on a small portion of the project area; DEIR Fig 2.7-1; and another one showing an "example" of fuel widening; DEIR Fig. 2.7-2; but these two maps are insufficient to convey the extent and precise locations of all the fuelbreaks to be constructed or widened.

Similarly, information on the locations of rare plants in relation to fuelbreak construction or widening is lacking. The Plan includes maps that depict locations of special status plant species, Figures 2-9 to 2-14, but the identity of the actual species is not stated. Again, it would have been entirely feasible to include this information in the DEIR.

Without the ability to discern which species occur where and how such populations might be affected by fuelbreak construction and/or widening, Marin CNPS is unable to intelligently evaluate the District's plans and mitigation measures. Indeed, it is impossible to determine whether the siting of new/widened fuelbreaks conform to the recent-developed scientific support for the concept of shifting vegetation treatment and other measures closer to the preserve perimeter and the WUI itself. Cohen, The Wildland-Urban Interface Fire Problem, Forest History Today (2008). A3-4

2. **The measures for mitigating impacts to rare plants and unique plant communities are impermissibly vague and inadequate**

CEQA requires that where impacts can be expected to be significant, the DEIR must set forth measures to mitigate such impacts to a less-than-significant level. "Formulation of mitigation measures shall not be deferred until some future time." CEQA Guidelines Section 15126.4.

The DEIR acknowledges that the BFFIP could cause significant, direct and indirect impacts on "any species identified as a candidate, sensitive or special status species in local or regional plans, policies, or regulations or by CDFW or USFWS." DEIR at ES-28. MM Biology-1. The mitigation measures set forth in the DEIR for such special status plants call for worker training, botanical surveys conducted within 5 years of the commencement of management activities, and avoidance of such species.

For species assigned a "low sensitivity ranking," disturbance is to be minimized but "complete avoidance is not necessary, as directed by the MMWD botanist." DEIR at ES-28 to 29; MM Biology-1.

A3-5

For moderately or highly sensitive species, perennial species are to be marked and avoided, and if not feasible, unspecified "hand methods" are to be employed to prevent damage or removal. DEIR at ES-29-31. MM Biology-2. As for rare annual plants, activities are to be timed when these species are senescent or have set seed and populations are to be monitored. If a population is found to be adversely impacted by management activities, "measures shall be taken" such as reducing the frequency of work or avoiding the area. No attempt to collect and distribute seed is called for. No criteria for successful restoration are stated. DEIR at ES-28 to 29; MM Biology-2.

Marin CNPS finds these measures inadequate. First, five-year old plant surveys may well be outdated.

Second, the DEIR fails to address the situation where a rare plant cannot be avoided, such as where it lies in the path of a new fuelbreak or the widening of an existing fuelbreak. The reference to "hand methods" is too vague to properly evaluate.

Third, the DEIR improperly downgrades the status of certain rare plants and fails to adequately mitigate impacts to these species. Mitigating Measure Biology-2 states that some special status plant species are "common" or of "low-sensitivity." Mount Saint Helena morning-glory is provided as an example of such low-sensitivity plants but the remainder of them are unidentified in the DEIR. The DEIR does not specifically call for avoidance of these plants and no specific mitigation plans will be prepared for these species. DEIR ES at 28- 29. The document cites no legal or scientific basis for this arbitrary approach and CNPS is not aware of any.

Despite the failure of the DEIR to identify the "low-sensitivity" species within the project area, the following species are deemed common in Table 3.3-5 and, based on the recent

DEIR for another MMWD project, likely included in this category: Tamalpais lessingia, Marin County navarretia, Tiburon buckwheat, Mt. Tamalpais Manzanita, Oakland star-tulip and serpentine reedgrass. The first 4 of these hold a California Rare Plant Rank (CRPR) of 1B, meaning they are rare and endangered throughout their ranges. The latter two species hold a CRPR of 4.2 and 4.3 respectively, meaning limited distribution and infrequent occurrence. See https://www.cnps.org/rare-plants/cnps-rare-plant-ranks. At least four of the rare plants documented in the project area—Mt. Tamalpais thistle, Tamalpais lessingia, Tiburon buckwheat and Tamalpais bristly jewelflower-- are endemic to the County or even to Mt. Tamalpais alone. *Marin Flora* at 40-41. Holding a CNPS or California Rare Plant Ranking, they are listed by CDFW and thus protected by CEQA and entitled to specific mitigation. [1] A3-5

Fourth, the mitigating measures are in some cases improperly deferred, particularly for populations of rare annuals that ongoing monitoring reveals to have been adversely affected by a management activity. In such cases, the DEIR states only that "measures shall be taken" such as reducing the frequency of work or avoiding the area." DEIR ES-31. This approach plainly violates the rule against deferring the formulation of mitigation measures. A3-6

Finally, expansion of fuelbreaks, even if it does not eliminate any individuals of rare or endangered species, may increase spread of invasive species, a result that goes contrary to one of the district's main goals. A3-7

Marin CNPS acknowledges that with a program EIR such as this DEIR, it may be permissible to leave the more specific identification of impacts and mitigation to later project-level review. CEQA Guideline Section 15168. Indeed, the District concedes that a "program EIR is most helpful in addressing subsequent activities if it analyzes the effects of the program as specifically and comprehensively as possible." DEIR 1-4. Unfortunately, however, the District apparently plans to implement most management activities set forth in the BFFIP without any project-level review. DEIR at 1-4. A3-8

---

[1] CEQA Guidelines Section 15380(d) is widely interpreted to require mitigation of California Rare Plant Rank1B species, even though such plants may not be listed under either the state or federal Endangered Species Act. Indeed, such species may properly be regarded as listed under state law because, while List B was initially created by CNPS, it is now under the aegis of a state agency, the California Department of Fish and Wildlife and has been renamed California Rare Plant Rank. Guidance by the CDFW states that any species listed by a government agency should be treated as rare under CEQA. http://www.resources.ca.gov/ceqa/guidelines/art20.html.

In sum, the DEIR's lack of specificity pertaining to the locations of fuelbreak projects, the species and plant communities to be adversely affected and the mitigation measures to be undertaken when avoidance is not feasible deprives Marin CNPS of a meaningful opportunity to evaluate it and renders the DEIR defective. A3-9

3. Marin CNPS supports the District's plan to use broadcast burning as a vegetation management tool and urges it to adopt the Limited Herbicide Alternative.

CNPS cautiously favors the use of controlled burns to suppress weeds and re-introduce fire to the landscape, provided appropriate measures are adopted to safeguard public safety and impacts to air quality. Ecologically, fire is an essential and highly positive force. It returns nutrients to the soil and regulates forest density. It creates conditions for plants to reproduce and influences species composition, favoring sun-loving conifers over shade-tolerant firs. It affects forest diseases, renews habitat for wildlife and fish, and increases the diversity of forest structure, species of plants, and age classes. Other fuel reduction techniques have significantly different ecological effects than fire[2] .We therefore support this aspect of the Plan. A3-10

At the same time, we urge the District to embrace the "limited herbicide alternative." DEIR at 4-1 to 4-48. Indeed, while the DEIR acknowledges that this alternative would be "more effective;" DEIR at 4-30 to 4-31; it rejects it because of "limited community acceptance." DEIR at 4-32. As stated in the DEIR, CEQA allows for rejection of alternatives on environmental, regulatory, technical and economic grounds; DEIR at 4-1; "limited community acceptance" does not fall under one of these categories.

Marin CNPS considers current community opposition to be misplaced because it is based largely on widespread aerial spraying of glyphosate to control weeds of food crops in the United States, which is not at all what the "limited herbicide" alternative of the DEIR describes; DEIR at 4-22 to 4-26. A3-11

The following key differences between the "limited herbicide" alternative and the situation causing public concern should be noted: First, the proposed use is for wildland weed control rather than for food crops. Second, herbicides are proposed for use in an Integrated Pest Management (IPM) program. IPM is the standard approach of public

[2] See Wilkin, K.M, L. C. Ponisio, D. L. Fry, C. L. Tubbesing, J. B. Potts, and S. L. Stephens. 2017, *Decade-long plant community responses to shrubland fuel hazard reduction. Fire Ecology* 13(2):105-136 (comparing effects of mastication and fire as fuel treatments).

P.O. Box 1408, Mill Valley, CA 94941-1408
www.marinnativeplants.org

lands managers for wildland weed context, controlling invasive plants. IPM, as defined in CNPS policy statements[3] and by the California Invasive Plant Council (Cal-IPC), calls for conservative, targeted use of herbicides for specific restoration projects to supplement mechanical and biological methods, based on effectiveness, efficiency, practicality, ecological impact, and safety.

Third, glyphosate is only one of several possible chemicals that are proposed for use, including some that are accepted in organic agriculture. Nevertheless, it is fully legitimate to include glyphosate among the possibilities. Cal-IPC, a recognized authority on control of invasive plants, continues to recommend the limited use of glyphosate for weed control despite its classification by the World Health Organization's International Agency for Research on Cancer as "probably carcinogenic to humans." Other agencies, including the US Environmental Protection Agency (EPA) and the European Food Safety Authority have recently reached different conclusions from the IARC; moreover, a review in the September 2016 issue of the journal Critical Reviews in Toxicology concluded that glyphosate is "unlikely to pose a carcinogenic risk to humans." https://www.cal-ipc.org/wp-content/uploads/2017/11/Cal-IPC-glyphosate-policy.pdf.[4] A3-11

Indeed, the IARC classification of glyphosate as "probably carcinogenic to humans" only designates a substance's carcinogenic *potential*; it does not consider actual exposures in real-world situations. "When they did consider exposure, the World Health Organization itself (through its Panel of Experts on Pesticide Residues in Food and the Environment) and the United Nation's Food and Agriculture Organization, in a joint meeting in 2016, concluded that 'glyphosate is unlikely to pose a carcinogenic risk to humans from exposure through the diet.'" https://www.cal-ipc.org/wp-content/uploads/2017/11/Cal-IPC-glyphosate-policy.pdf.

For these reasons, Cal-IPC "supports the use of glyphosate in invasive plant management as part of an of Integrated Pest Management (IPM) approach. When using glyphosate according to the label, with appropriate personal protective equipment and best practices, glyphosate is low-risk for wildlife, applicators and the public." https://www.cal-ipc.org/wp-content/uploads/2017/11/Cal-IPC-glyphosate-policy.pdf.

[3] CNPS official policy statements can also be found at http://www.cnps.org/cnps/conservation/pdf/IWM_policy.pdf and http://cnps.org/cnps/conservation/pdf/Herbicide policy.pdf).

[4] The US Environmental Protection Agency also rejected the notion that glyphosate is an endocrine-disrupter. http://www2.epa.gov/sites/production/files/2015-06/documents/glyphosate-417300_2015-06-29_txr0057175.pdf.

Fourth, proposed application methods are different than those most commonly used in agriculture. The DEIR describes topical application to freshly cut woody stumps or gashes in bark, preliminary mowing of plants before low-height spraying of small areas with backpack sprayers, along with other specific targeted techniques.

Finally, the DEIR proposes extensive safety regulations to minimize risks to applicators, visitors, water and wildlife. MMWD has been struggling to manage its invasive plants without herbicide, having discontinued such use in 2005. By 2008, French broom had choked all its firebreaks, requiring an expensive mowing regime. In 2008, the District estimated that, without herbicides, its IPM program will cost $7.8 million annually compared to $2.9 million, if herbicide use is included. And even with such high costs, the October, 2014 issue of *Bay Nature* quoted MMWD staff stating that, despite the efforts of volunteer and paid labor, French broom was expanding faster than they could remove it. MMWD should not be rejecting a safe, effective tool to address this serious ecological problem. A3-11

In practice, IPM programs are continually adjusted as new information and techniques become available. Rejection of all chemical use precludes this adjustment. Furthermore, it goes directly contrary to one of the main procedural characteristics of the plan, which is adaptive management. DEIR at 2-60. Including judicious use of herbicides in an IPM plan is the wisest and most fiscally prudent choice for reducing environmental damage and fire danger resulting from invasive plants on County lands.

In summary, Marin CNPS finds that the DEIR for the BFFIP should

1. specify the locations of new fuelbreaks and those to be widened, identify the locations of special status species by reference to the species in question, acknowledge the possible negative effects of completing the proposed fuelbreak system and include appropriate mitigation; A3-12

2. eliminate the category of "common" or "low-sensitivity" rare plants, remedy the vagueness of mitigation measures for rare plants, and avoid deferral of mitigation measures to a future date; A3-13

3. Include sufficient detail on all management actions and mitigation measures so as to eliminate the need for project-level review; A3-14

4. include additional evidence supporting limited broadcast burning; A3-15

5. adopt the limited herbicide use alternative as the one most likely to meet the district's goals with the minimum environmental impacts. A3-16

Thank you for the opportunity to comment.

Sincerely,

Paul DaSilva, Director
Marin Chapter, California Native Plant Society

Carolyn Longstreth, Director
Marin Chapter, California Native Plant Society

California Native Plant Society- Marin Chapter
P.O. Box 1408, Mill Valley, CA 94941-1408
www.marinnativeplants.org

8

2.3.3 Letter A3: Carolyn and Paul DaSilva, Marin Chapter of California Native Plant Society

Response to Comment A3-1

The commenter states that the Marin Chapter of CNPS has 10,000 members dedicated to protecting native plants and their habitats. The comment about the role of the California Native Plant Society (CNPS) is noted.

Response to Comment A3-2

The commenter notes that the DEIR's description of the project is inadequate because it fails to mention where fuelbreak construction projects will occur related to the locations of special-status plants and that mitigation measures for rare plants are vague. The comment also states that the DEIR improperly categorizes special-status plants as having high or low sensitivity. Refer to **Master Response 1: Definition and Location of New and Expanded Fuelbreaks**. Refer to **Master Response 3: Special-Status Plants** for a response to the comments pertaining to special-status plant species mitigation measures and mapping information available in the EIR. Refer to **Master Response 6: Limited Use of Herbicides Alternative** for a discussion of the herbicide alternative and the assessment of the environmentally superior alternative per CEQA.

Response to Comment A3-3

The commenter states that the DEIR fails to disclose where specific fuelbreak construction or widening projects are to be undertaken. The Draft EIR adequately identifies and focuses on the significant effects of the program on the environment. The biological setting and impacts are presented in Section 3.3 Biological Resources, including over 140 pages of detail. The section describes the various biological resources found in the plan area (Section 3.3.3 on pages 3.3-3 through 3.3-66 of the Draft EIR), and the physical changes that would occur as a result of implementation of the plan (Section 3.3.6 on pages 3.3-73 through 3.3-123 of the Draft EIR), including alterations to ecological systems and in population distribution.

The commenter states that the Draft EIR does not identify special-status plant species locations in relation to new fuelbreaks. Refer to **Master Response 1: Definition and Location of New and Expanded Fuelbreaks** for a discussion of new and expanded fuelbreak locations as shown in Figures 2.6-1 through 2.6-4 and Figure 2.7-1. Refer to **Master Response 3: Special-Status Plants** for a discussion as to how the impacts from creation of new and expanded fuelbreaks on special-status plants is adequately addressed in the Draft EIR. The Draft EIR focuses on identification of rare plants during pre-work surveys and avoidance based on the species found and its life form, as defined in MM Biology-2. Minor revisions to MM Biology-2 have been made and are shown in Chapter 3 of this Final EIR.

Response to Comment A3-4

The commenter states that it is impossible to determine whether the siting of new fuelbreaks conforms to the recently developed scientific support for the concept of shifting vegetation treatment closer to the preserve perimeter. Section 3.4 on pages 3-17 through 3-18 of the March 2019 Draft BFFIP discusses how the District assessed fuelbreak needs and locations, based in part on an assessment of risks and the location of the Wildland Urban Interface (WUI). **Master Response 1: Definition and Location of New and Expanded Fuelbreaks** provides additional response to concerns over the location of new and expanded fuelbreaks.

Response to Comment A3-5

The commenter notes that the BFFIP could cause significant direct/indirect impacts on special-status plant species. The commenter also notes that the DEIR fails to address the situation where a rare plant cannot be avoided, such as in the path of a new fuelbreak. The commenter also states that the DEIR improperly downgrades the status of certain rare plants and fails to provide proper mitigation. Refer to **Master Response 3: Special-Status Plants** for a discussion of MM Biology-2 and how the measure adequately addresses impacts on special-status plant species with minor revisions to strengthen the measure. Potential impacts on special-status plants with known rarity or declining populations and special-status plants with CRPR rank of 1B or 2 and some rank 4 species that are known rare are addressed with the minor revisions to MM Biology-2, presented in Chapter 3. Tamalpais lessingia, Marin County navarretia, Tiburon buckwheat, Mt. Tamalpais Manzanita, Mt. Tamalpais thistle, and Tamalpais bristly jewelflower, as identified by the commenter, are CRPR rank 1B and are addressed by the mitigation measure. MM Biology-2 has been revised in Chapter 3 to indicate the process required should a perennial or annual population of rare plant not be avoidable. Oakland star-tulip was not added to the mitigation as it is a rank 4.2 but is "abundant and stable on the Watershed," (see page 3.3-31 of the Draft EIR). Serpentine reed, also, was not added to the mitigation. Serpentine reed grass is a rank 4.3 but is "abundant, stable, and widespread through serpentine chaparral habitats in the Watershed" (see page 3.3-31 of the Draft EIR).

Response to Comment A3-6

The commenter states that the mitigation is sometimes improperly deferred, particularly for rare plants that require ongoing monitoring. An agency may defer committing to specific mitigation measures when it approves a project if the measures that will be considered subsequently are described and performance criteria are identified (*Sacramento Old City Assn. v. City Council* (1991) 229 Cal.App.3d 1011; see CEQA Guidelines, § 15126.4, subd. (a)(1)(B)). Deferred mitigation is a complex concept under CEQA. Per a paper by Curtis Alling (2011) on the topic, he describes,

> It is adequate to recognize a significant effect, adopt a measure that commits the lead agency to mitigate, and describe the specific performance criteria for mitigation, if the plans, design details, or precise means to mitigate are not practical to define at the time of project approval… The commitment to mitigate should be accompanied by a list of potential approaches to achieve the avoidance or lessening of the significant effect to demonstrate that the eventually selected measures are reasonably expected to be feasible and effective.

MM Biology-2 describes the performance criteria and a list of approaches to achieve avoidance or lessening of the significant effects. Performance criteria include determining if habitat is present, consulting the GIS database to see what known populations are in the area of work, conducting a survey if the area has not been surveyed within the last five years, and then flagging or demarcating individual plants or populations for avoidance, establishing a 100-foot buffer, and requiring BMPs to minimize the potential for spread of invasive species. Criteria specific to perennial sensitive plants and annual sensitive plants are also defined, including no net loss of an annual species and the methods to employ if an individual or population must be removed. The measure also requires a monitoring plan with standards to meet and actions to take if standards are not met.

To further solidify the approach to demonstrate that the measure can reduce effects, the text of MM Biology-2 has been revised to indicate that, at a minimum, one of the options presented would be taken should a population decrease following vegetation management activities.

Response to Comment A3-7

The commenter states that the expansion of fuelbreaks may increase spread of invasive species. The purpose of the fuelbreak is to provide an area of defense and improve containment in the event of a wildfire, which is consistent with the District's main goals for the BFFIP. Once a fuelbreak is created, it would be maintained per MA-20, including to reduce invasive species in the new fuelbreak.

Response to Comment A3-8

CEQA Guidelines

The commenter states that the District will implement most management activities set forth in the BFFIP without any project-level review. CEQA Guidelines section 15168, subdivision (c), applies to the use of a program EIR for later activities. If a later activity would have effects not examined in the Program EIR, an Initial Study would need to be prepared leading to an EIR or a Negative Declaration. (CEQA Guidelines, § 15168, subd. (c)(1).) See **Master Response 7: Benefits of the BFFIP and Program EIR** for a discussion of when a Program EIR applies and should be prepared. A Project Environmental Checklist form has been prepared and is presented in Appendix A of this Final EIR. The form allows the District to provide the substantial evidence necessary to document whether or not future activities are covered under the Program EIR or if additional review is required.

Level of Information Available

Chapter 2 Project Description, with incorporation of the BFFIP by reference, provides detailed project information. Each management action is described with annual performance criteria clearly identified in Table 2.7-1 of the Draft EIR, with activity ramping up in intensity from Year 1 through Year 5. The types of tools and techniques proposed to implement the performance criteria for each management action are identified in Table 2.9-1 of the Draft EIR. A clear description of each tool and technique proposed for use as part of the BFFIP is provided under Section 2.9 of the Draft EIR. The level of detail available for each management action generally allows a project-level analysis for all but MA-26 and MA-27. The analyses of MA-26 and MA-27 are conducted using the data available at the time this EIR was prepared. The specific actions that may occur under MA-26 and MA-27 have not been identified to the same level of detail as the other management actions. When specific activities are proposed for either management action, the District would perform project-level environmental review, presented on Project Environmental Checklist (Appendix A of this Final EIR) to document whether or not future activities are covered under the Program EIR or if additional review is required. See **Master Response 7: Benefits of the BFFIP and Program EIR** for more information. Prior to approving site-specific activities under these management actions, the District would evaluate the selected site by completing the checklist, which has been adapted from the Initial Study checklist in the CEQA Guidelines, and from the information in this Program EIR. The Project Environmental Checklist would be used to determine whether the activity proposed under MA-26 or MA-27 is within the scope of the analysis in this Program EIR. Subsequent environmental review would be conducted if determined to be necessary. The checklist would also identify those mitigation measures set forth in this Program EIR that are relevant to the activity under consideration. The analysis of management actions is comprehensive using the level of detail known at the time of the EIR. For the majority of actions proposed, the analysis is project level and will enable the District to conduct the actions without further environmental review.

Response to Comment A3-9

The commenter states that the DEIR lacks specifics pertaining to locations of fuelbreak projects and the species and communities to be adversely affect as a result. Refer to **Master Response 1: Definition and Location of New and Expanded Fuelbreaks** for locations of new fuelbreaks within the EIR. Refer to **Master Response 3: Special-Status Plants** for information about the data and maps available for special-status plant species in the EIR.

Response to Comment A3-10

The commenter states that CNPS supports the use of controlled burns to suppress weeds and re-introduce fire to the landscape. The support for prescribed burning is noted. Appropriate mitigation measures were included in the Draft EIR to safeguard the public. These measures address impacts on air quality, public safety, and fire hazards and include MM Air-1, MM Air-4, MM Hazards-4, and MM Hazards-5.

Response to Comment A3-11

The commenter notes that the CNPS supports the Limited Use of Herbicides Alternative. The support for the Limited Use of Herbicides Alternative is noted. Refer to **Master Response 6: Limited Use of Herbicides Alternative** for a discussion of the herbicide alternative and why it was not identified as the environmentally superior alternative. While the Draft EIR identifies that the use of herbicides had limited community acceptance, the alternative was not identified as the environmentally superior alternative because it did not address the significant and unavoidable air quality impacts of the BFFIP (from prescribed burning) and it could introduce new impacts related to health hazards, the extent of which are likely less than significant but not well known. The numerous points regarding conflicting conclusions on glyphosate toxicity and carcinogenic potential as well as on IPM are noted; however, these points raised do not merit changing the analysis presented in the Draft EIR. These points do not change the fact that some impacts from herbicides could occur that would otherwise not occur under the BFFIP, as identified in the master response. The commenter's identification of the increased costs associated with not using herbicides is noted and can be considered by the Board when evaluating whether to approve the BFFIP or an alternative. CEQA, however, is not about analysis of economic impacts per se (PRC § 21080(e)(2) ['evidence of social or economic impacts which do not contribute to, or are not caused by, physical impacts on the environment' are beyond the scope of CEQA]; see also PRC § 21082.2(c); Guidelines § 15384). The economic impacts of a project are only subject to CEQA if those financial impacts cause physical impacts. Added costs for manual or mechanical removal over herbicide usage do not have physical impacts on the environment. The BFFIP is designed to reduce the impacts of invasive species and improve ecological health on the watershed, even without herbicides. The plan includes criteria and goals to be addressed to the District's Board annually. The adaptive management aspect of the program will allow for reassessment of methodology, noting that herbicides would not be included without further environmental review and District Board approval.

The commenter is incorrect that the community opposition to glyphosate use is based on widespread aerial spraying in agriculture. Community opposition in Marin County is highly specific to the introduction of any herbicide into the environment due to the potential for endocrine disruption and carcinogenicity, among other health effects. This opposition that was specific to limited use of herbicide was extensively expressed and documented when the 2012 Draft of the Wildfire Protection and Habitat Improvement Plan (WPHIP) was released.

The BFFIP does not preclude the future use of herbicides by the District; however, any future proposals for herbicide use are explicitly not covered under the BFFIP and BFFIP EIR. Use would require additional review under CEQA and District Board approval.

Response to Comment A3-12

The commenter notes that the BFFIP DEIR should specify locations of new fuelbreaks and those to be widened, identify special-status plants in reference to the locations, and provide adequate mitigation to address the species. Refer to **Master Response 1: Definition and Location of New and Expanded Fuelbreaks** and **Master Response 3: Special-Status Plants,** which identify how the Draft EIR addresses new and expanded fuelbreaks and their impacts on special-status plants.

Response to Comment A3-13

The commenter notes that the BFFIP DEIR should eliminate "low sensitivity" rare plants and vague mitigation addressing them. Refer to **Master Response 3: Special-Status Plants** for a discussion of the ranking system used for special-status plant species and the mitigation measures required to address impacts on these species.

Response to Comment A3-14

The commenter notes that the BFFIP DEIR should include sufficient detail on management actions and mitigation to eliminate need for project review. Refer to Response to Comment A3-8 for the requirements for a Program EIR and the level of detail available at the time the EIR was prepared.

Response to Comment A3-15

The commenter notes that the BFFIP DEIR should include additional evidence supporting limited broadcast burning. Broadcast burning is included in the BFFIP and addressed throughout the EIR. The impacts of broadcast burning are analyzed as applicable under each resource topic in the EIR. Broadcast burning is becoming an important tool for land managers to address fuel loading and habitat enhancement. The emissions and carbon release from broadcast burning areas of a natural landscape under controlled conditions would be considerably less than the emissions if the area were subject to a wildfire. The benefits of broadcast burning may outweigh the cost of temporary significant emissions during the burn. The use of broadcast burning is sufficiently supported in the Draft EIR.

Chapter 4 Alternatives to the Proposed Plan, as presented in the Draft EIR, provides a detailed analysis of the Refocused Effort Alternative, which limits broadcast burning to 22 acres or less to treat weeds such as starthistle. No broadcast burning would occur in grasslands and oak woodlands for habitat enhancement. Although this alternative would reduce some direct impacts, other impacts would be greater than for the proposed plan. The alternative would only marginally meet some of the plan’s main objectives. The Refocused Effort Alternative was not identified as environmentally superior to the proposed plan.

Response to Comment A3-16

The commenter notes that the BFFIP DEIR should adopt the Limited Use of Herbicides Alternative. The support for the Limited Use of Herbicides Alternative is noted. Refer to **Master Response 6: Limited Use of Herbicides Alternative** for a discussion of the herbicide alternative and why it was not identified as the environmentally superior alternative.

WATERSHED
ALLIANCE OF MARIN

Comment Letter A4

June 17, 2019

Shaun Horne, Natural Resources Manager
Marin Municipal Water District
220 Nellen Avenue Corte Madera, CA 94925
bffipeir@marinwater.org

RE: The MMWD Draft Program Environmental Impact Report for the Biodiversity, Fire, and Fuels Integrated Plan

Dear Mr. Horne and MMWD Board:

The Watershed Alliance of Marin (a 501C3) agrees wholly with the comment letter submitted by the Sierra Club Marin Group regarding the Biodiversity, Fire and Fuels Integrated Plan DEIR. A4-1

Our only addition is that we would also like to see that there is inclusion of managing fuel loads with carbon sequestering char production since it would be a great service to the climate and the soils. A4-2

Furthermore, under existing statutes, we support that the Federated Indians of Graton Rancheria will continue to be consulted on all relevant cultural matters as in Appendix F pages 10 and 12. "Consultation with FIGR is ongoing as the BFFIP progresses." A4-3

"Seventy-five (75) resources have been formally recorded or identified within the three watersheds (61 historic sites, 13 prehistoric sites, 1 prehistoric/historic site). Only one appears to have been formally evaluated although several sites appear to be eligible." We believe that those sites must be evaluated wherever possible and prior to any work consideration under best management practices. One site that must be evaluated is the Master Sargent Cypress - the tallest in the world - near Barth's retreat. All sites, particularly where living legacy plants, trees and animals exist must be evaluated wherever possible and prior to any work. A4-4

Thank you for your consideration.

Sincerely,

Laura Chariton

Laura Chariton, President, Watershed Alliance of Marin,

M.A. Riparian Policy & Environmental Restoration

watermarin@comcast.net Watermarin.org 446 Panoramic Hwy, Mill Valley, CA 94941 (415) 234-9007

2.3.4 Letter A4: Laura Chariton, Watershed Alliance of Marin

Response to Comment A4-1

The commenter states that the Watershed Alliance of Marin agrees with the Sierra Club's comment letter on the DEIR. The comment regarding the role of the Watershed Alliance of Marin is noted. Refer to responses to comments under Letter A2, Sierra Club Marin Group.

Response to Comment A4-2

The commenter states that the only addition to the Sierra Club's comment letter would be to include managing fuel loads with carbon sequestering char production. The recommendation to manage fuel loads with carbon sequestration char production is noted. Refer to Response to Comment A2-3 for a summary of the carbon sequestration analysis presented in the Draft EIR. Biochar is an organic charcoal material that is the final product of pyrolysis, or high temperature burning of agricultural biomass without the presence of oxygen. This type of activity is outside the scope of the BFFIP, which is focused on vegetation management.

Response to Comment A4-3

The commenter states support that the Federated Indians of Graton Rancheria will continue to be consulted. The comment is noted. Section 3.4.2.3 of the Draft EIR discusses the tribal outreach and consultation that has been conducted to date.

Response to Comment A4-4

The commenter states that all sites must be evaluated that have had recorded sensitive resources. The comment also states that the Master Sargent Cypress site should be evaluated prior to work commencing. As discussed under Response to Comment A2-27, impacts on cultural resources from implementation of the BFFIP are analyzed in Section 3.4 Cultural and Tribal Cultural Resources of the Draft EIR. It is acknowledged that cultural surveys have only been conducted in discrete locations and not for most of the plan area. MM Cultural-2, as stated in the Draft EIR, requires review of confidential GIS data prior to conducting work to determine if the area has been surveyed previously and whether any resources were found. Any resources that have not been evaluated would be avoided or, if they cannot be avoided and the activity would have an impact on the resource, they would be evaluated. If the GIS data shows that the areas where soil disturbance below the surface through use of heavy equipment or burning is proposed have not been previously surveyed, a pre-activity cultural resources survey would be conducted by a qualified archaeologist or cultural resources specialist in accordance with industry standards prior to performing work, and any resources found would either be avoided or evaluated and data collected per a Cultural Resources Management Plan. Implementation of this measure would either avoid impacts to unevaluated or known eligible resources or would include evaluation and data collection for unavoidable resources, effectively mitigating impacts.

Section 3.3 Biological Resources of the Draft EIR analyzes impacts on the sensitive Sargent cypress woodland community, within which the Master Sargent Cypress referred to by the commenter is found. With implementation of mitigation measures, impacts on the sensitive Sargent cypress woodland community would be less than significant. The tree has not been identified as a cultural resource but would be protected under biological resource mitigation.

Comment Letter A5

June 19, 2019

Shaun Horne, Natural Resources Manager
bffipeir@marinwater.org

RE: Comments on BFFIP and draft EIR

Mr. Horne,

Since Friends of Corte Madera Creek Watershed was formed in 1995, we have followed the management of the District's watershed with great interest. Tributaries to Corte Madera Creek drain portions of the District's watershed and its condition has major impacts on Corte Madera Creek. We have supported rate increases so that the watershed can be better managed. We are eager to see a vegetation management plan that can be successfully implemented to reverse the spread of invasive plants and lead to improved biodiversity, forest health, and water yield. A5-1

BFFIP Comments

Implementation of Integrated Pest Management

The Environmental Protection Agency defines IPM this way:

> Integrated Pest Management (IPM) is an effective and environmentally sensitive approach to pest management that relies on a combination of common-sense practices. IPM programs use current, comprehensive information on the life cycles of pests and their interaction with the environment. This information, in combination with available pest control methods, is used to manage pest damage by the most economical means, and with the least possible hazard to people, property, and the environment.

Note that an IPM program does not rule out herbicide use. It calls for using the method that is economically feasible and poses the least hazard to people, property, and the environment. In some situations, that method is herbicide application. A good example would be an infestation of dense invasive plants in an inaccessible location where it cannot be treated manually without enormously expensive labor costs. Another example is an infestation of Japanese knotweed (*Fallopia japonica*), which has been found just downstream of District lands; the only effective method of controlling this destructive pest is by applying herbicides. A5-2

The District's proposed BFFIP is not a robust IPM program. Goal 3, Approach 3.5 suggests updating the District's IPM policies and techniques in response to new information. MA-1 calls for an annual update of invasive species maps and MA-18 calls for a redo of the comprehensive invasive species maps every five years. These updates will provide a record of the effectiveness of the BFFIP in controlling invasive species. We expect that the Board will revisit its no-herbicide policy to include a robust IPM approach if invasives continue to spread. By making a political decision, not one based on well-established principles of vegetation management, to not allow the use of any herbicides on the watershed, the board has compromised the condition of the watershed and ultimately our water supply which relies on the watershed.

PO Box 415 • Larkspur CA 94977 • 415-456-5052 • info@friendsofcortemaderacreek.org

Friends Comments on BFFIP and EIR
June 19, 2019
Page 2

Performance Criteria
Table ES-3 (a repeat of Table 6-1) quantifies performance criteria after 5 years for vegetation management actions MA-20 through MA-27. The BFFIP was written several years ago, but its release was delayed numerous times. Have these performance criteria been increased to reflect the spread of invasives in the intervening years? If not, they should be updated to compensate for degradation that has occurred during delays. We expect that the BFFIP will be revised if these targets are not met, including the addition of herbicide use. A5-3

Costs
Chapter 7 identifies costs for the first 5 years of BFFIP implementation (totals corrected):

	Year 1	Year 2	Year 3	Year 4	Year 5	Total
Inventorying, monitoring, and planning management actions	137,700	107,500	161,100	246,400	283,600	**936,300**
Vegetation management (fuel breaks)	478,600	631,600	670,400	721,000	801,600	**3,303,200**
Vegetation management (MA-22 thru MA-27)	1,294,500	1,503,180	1,702,080	1,822,500	1,976,778	**8,299,038**
Initial capital cost	200,000	200,000	200,000	200,000	200,000	**1,000,000**
Total	**2,010,800**	**2,542,280**	**2,713,580**	**2,989,900**	**2,861,978**	**13,538,538**

A5-4

The preliminary FY 2020 and 2021 operating and capital budget dated 2/19/2019 allocates approximately $18.6 million in 2020 and $19 million in 2021 for the Facilities and Watershed Division. Therefore, management of the watershed is allocated ±12% of the Facilities and Watershed budget for the first two years of implementation of the BFFIP. More should be spent on vegetation management.

There is no estimate of costs for the limited herbicide alternative. However, the 2012 draft of the vegetation management plan, renamed the Wildfire Protection and Habitat Improvement Plan (WPHIP) at the beginning of the EIR process, presented a comparison of the cost and effectiveness of two alternatives, one without any herbicide use and one with modest initial herbicide use. The annual cost to "fully implement" the 2012 plan without herbicides would have been $5.6 million. The annual cost if herbicides were used as part of the initial treatment would have been $1.6 million (2012 dollars). Put another way, the earlier plan estimated that accomplishing "full implementation" without herbicides would have cost 350% more than using herbicides to accomplish the same goal. However, it is important to note that the 2012 draft WHPIP estimates that while full implementation using herbicides would result in meeting 100% of the eleven stated targets, results were much worse without herbicides. If no herbicides were used only four tasks would have met 100% of the target, with one as low as 18% and three more less than 50%. This hardly constitutes full implementation. It is likely that absent the limited use of herbicides, full implementation could never have been achieved. A5-5

Friends Comments on BFFIP and EIR
June 19, 2019
Page 3

The draft EIR should be revised to include a cost analysis comparing effectiveness and cost of the proposed plan in the BFFIP and the limited herbicide alternative. A5-5

Fishery Resources
Part J of Board Policy No. 7, dated 9-01-10, deals with Fishery Management. It states in part:

> Streams: The District will take actions to protect native fishery resources, in streams within the District's sphere of influence, consistent with California public trust doctrine and Fish and Game Code. The District will be an active partner in stream protection and enhancement efforts that other agencies and groups are pursuing in streams within the District's sphere of influence. The District's sphere of influence includes those streams that are directly affected by the District's land or water management activities.

A5-6

Although the District is required by the California State Water Resources Control Board to implement fishery protection and enhancement activities in Lagunitas Creek, other streams do not receive any attention and the District's policy is ignored with respect to tributaries of Corte Madera Creek that are occupied by or provide habitat for *Oncorhynchus mykiss* (tributaries to San Anselmo Creek, Ross Creek, and Tamalpais Creek). These creeks and the fish and other wildlife they support would benefit from enhancements to riparian vegetation and some are directly impacted by the District's activities.

Draft EIR Comments

Impacts Analysis
The Deferred Action Zone is defined in section 2.6.3.5 as follows:

> This zone is characterized by the dominance of large, persistent populations of perennial weeds, hard to access stands of diseased trees, lack of special-status species, and diminished ecosystem function. Neither the District's wildfire goals nor ecological goals are likely to be achievable in these areas without very intensive and repeat treatment, making it a lower priority than in areas where success can be more readily attained. Therefore, the strategy is to defer large-scale action but contain weeds where strategically possible.

Ignoring these degraded areas should be considered a significant impact of the proposed BFFIP. This impact makes the limited use of herbicides even more compelling as much of this area could be effectively treated with herbicides. A5-7

Section 3.3.3.3, a discussion of invasive species, provides an excellent summary of the impacts of invasive plants, especially broom, on various vegetation types on District lands. However, the impact analyses in section 3.3.6 only address the impacts of specific management actions. There is no discussion of the negative impacts of inaction. This is a glaring omission.

Friends Comments on BFFIP and EIR
June 19, 2019
Page 4

Alternatives Analysis
The alternatives analysis includes a discussion of the Limited Use of Herbicides Alternative, stating as follow on pages 4-30 and 4-31 of the Draft EIR:

> This alternative reduces several environmental impacts, all of which are either less than significant or mitigable under the proposed plan. It does not reduce the potentially significant and unavoidable impacts on air quality and GHGs from prescribed burning.
>
> This alternative may result in a more effective program, as herbicide use would allow more areas to be treated since it requires less equipment and workers to implement and less repeated work to remove weeds. A more effective plan could further reduce fire hazards and improve biological diversity and habitat across the plan area. While the proposed limited herbicide use under this alternative has many benefits, it introduces several new potential effects that would not occur under the proposed plan. These effects include exposure risks to animals, to humans including applicators, and to water quality. None of the effects would be significant given the limited use of herbicides and the numerous application restrictions, but some level of risk and impact would remain that would not occur under the proposed plan. This alternative would meet all of the plan objectives.

This alternative is superior to the proposed BFFIP and should be adopted by the Board. A5-8

Additions and Corrections

A foothill yellow-legged frog (*Rana boylii*) population has been found in Marin County Park's Cascade Canyon Open Space Preserve, downstream of District lands. BFFIP Appendix D, page D-15, should be updated to include this new information. A5-9

Steelhead trout (*Oncorhynchus mykiss*) are stated as occupying Larkspur Creek. This sighting is very old, and it is not likely that there is a viable population in Larkspur Creek. A5-10

Sincerely,

Sandra Guldman

Sandra Guldman
President

2.3.5 Letter A5: Sandy Guldman, Friends of the Corte Madera Creek Watershed

Response to Comment A5-1

The commenter states that Friends of Corte Madera Creek Watershed is eager to see a vegetation management plan that can be successfully implemented. The comment is noted regarding the role of the Friends of Corte Madera Creek Watershed's purpose and role.

Response to Comment A5-2

The commenter states that the definition of IPM calls for using the method that is economically feasible and uses herbicide application. The comment is noted regarding the definition of IPM. The BFFIP is not a true IPM program, as the commenter notes, because it does not include the judicious use of herbicides. The Limited Use of Herbicides Alternative would include the judicious use of herbicides; however, it was not the environmentally superior alternative, as explained in **Master Response 6: Limited Use of Herbicides Alternative**. While the plan is not a true IPM program, the BFFIP is designed to reduce the impacts of invasive species and improve ecological health on the watershed, even without herbicides. The plan includes criteria and goals to be addressed to the District's Board annually. The adaptive management aspect of the program will allow for reassessment of methodology. The BFFIP does not preclude the future use of herbicides by the District; however, any future proposals for herbicide use are explicitly not covered under the BFFIP and BFFIP EIR. Use would require additional review under CEQA and District Board approval.

Response to Comment A5-3

The commenter questions if the performance criteria in the BFFIP have increased to reflect the spread of invasive species in the years that the BFFIP was delayed in getting released. The performance criteria for each management action as presented in the March 2019 Draft BFFIP was revised and updated, as appropriate, since preparation and release of the September 2016 Draft BFFIP. The BFFIP includes annual revision and adaptive management to move the plan towards meeting the targets. See Section 7.3 of the BFFIP, page 7-7, which states the following:

> If these targets are not being reached, the reasons will be documented in the Annual Board Reports and the success criteria may need to be modified or levels of effort to implement the Plan increased to more closely align what is actually being accomplished with what is planned. A balance between the costs and the benefits is inherently part of the evaluation and adaptive management strategy.

Herbicide use, if added, would require additional review under CEQA and District Board approval, as stated in Response to Comment Af-2. No additional changes are needed.

Response to Comment A5-4

The commenter states that more funding should go to vegetation management. The comment identifying the costs from Chapter 7 of the BFFIP is noted.

Response to Comment A5-5

The commenter states that there is no estimate of costs for the limited herbicide alternative. The comment also notes that the DEIR should be revised to include a cost analysis comparing effectiveness and cost of the proposed plan in the BFFIP and the limited herbicide alternative. The comment is noted regarding costs of the limited herbicide alternative. According to the CEQA Guidelines the "EIR shall include sufficient information about each alternative to allow meaningful evaluation, analysis, and comparison with the proposed project." The description and analysis of an alternative does not need to be as robust as the proposed project (Section 15126.6; County of Inyo v. City of Los Angeles (1981)124 Cal.App.3d 1). Chapter 4 Alternatives to the Proposed Plan of the Draft EIR evaluates the alternatives with respect to consistency with plan objectives, feasibility, and environmental effectiveness. The effectiveness of each alternative retained for analysis, including the Limited Use of Herbicides Alternative, to meet the plan objectives is summarized in Table 4.5-1 of the Draft EIR and within the analysis for each alternative. The Limited Use of Herbicides Alternative was found to meet plan objectives of reducing weeds and enhancing biodiversity while allowing for adaptive management.

See Response to Comment A3-11 for an explanation as to how costs are considered (or not considered) under CEQA. The commenter's identification of the increased costs associated with not using herbicides are noted and can be considered by the Board when evaluating whether to approve the BFFIP or an alternative. CEQA does not require analysis of economic impacts per se (PRC § 21080(e)(2) ['evidence of social or economic impacts which do not contribute to, or are not caused by, physical impacts on the environment' are beyond the scope of CEQA]; see also PRC § 21082.2(c); Guidelines § 15384). The economic impacts of a project are only subject to CEQA if those financial impacts cause physical impacts, which is not the case here, and thus the economics of implementing the BFFIP is not considered in the EIR.

Response to Comment A5-6

The commenter states that the District should implement fishery protection and enhancement activities in the tributaries of Corte Madera Creek as they are currently not addressed. The BFFIP provides protection to riparian habitat and would allow only limited activities to occur in riparian habitat. Refer to Response to Comment A2-25. Impact Biology-2 in Section 3.3 Biological Resources of the Draft EIR provides a clear understanding of the types of activities that could and would not occur in riparian areas. Vegetation removal in riparian habitat would generally consist of invasive species removal. Broadcast burns could occur within riparian habitats, but MM Geology-1 prohibits broadcast burning within a 50-foot buffer around perennial and intermittent streams when the broadcast burn is proposed on a slope greater than 30 percent and upslope of the stream (and, therefore, associated riparian habitat) so that impacts would be avoided. Riparian corridors would not be significantly and negatively impacted by implementation of the BFFIP.

The Draft EIR also addresses indirect impacts to steelhead through sedimentation of streams. A description and analysis of steelhead (*Oncorhynchus mykiss irideus*) is provided in Section 3.3 Biological Resources of the Draft EIR. It is noted that steelhead are known to occur not just in the mainstem of Lagunitas Creek and tributaries but also other creeks, including Corte Madera

Creek. Direct and indirect impacts on steelhead from implementation of the BFFIP are analyzed under Impact Biology-1 in text and in Table 3.3-8 of the Draft EIR.

Response to Comment A5-7

The commenter notes that disregarding the Deferred Action Zone should be considered a significant impact under the BFFIP. Refer to **Master Response 2: Ecosystem and Fuels Deferred Action Zone** for a description of the Ecosystem and Fuels Deferred Action Zone. Maintenance activities occur in this zone under existing conditions and would continue to occur following implementation of the BFFIP. No change in the management of this zone would occur compared to existing conditions as a result of plan implementation. Any environmental effects associated with the existing weeds and diseased trees are part of the baseline conditions for the EIR (CEQA Guidelines Section 15125). No new impacts would occur as a result of the BFFIP that need to be analyzed in the EIR.

Response to Comment A5-8

The commenter states that the Limited Use of Herbicides Alternative is superior to the proposed plan. The commenter's support for the Limited Use of Herbicides Alternative is noted. Refer to **Master Response 6: Limited Use of Herbicides Alternative** for a discussion of the herbicide alternative and why it was not identified as the environmentally superior alternative.

Response to Comment A5-9

The commenter states that the BFFIP should be updated to include the new population of foothill yellow-legged frog discovered in Marin County Park's Cascade Canyon Open Space Preserve. Surveys for foothill yellow-legged frog have recently been conducted on Marin County Parks and District lands but are currently unpublished. Some additional information about sightings from these recent studies has been added to Table 3.3-6 as shown in Chapter 3 of this Final EIR.

Response to Comment A5-10

The commenter noted that the steelhead trout occupying Larkspur Creek is an old data point and that there is unlikely to be a viable population currently. Larkspur Creek is considered a stream that Central California coast steelhead could occupy (Ettlinger, 2019). No change has been made.

June 19, 2019

Comment Letter A6

MARIN CONSERVATION LEAGUE
Protecting Marin Since 1934

Shaun Horne, Manager Natural Resources
Marin Municipal Water District
220 Nellen Avenue
Corte Madera, CA 94925-1169
By Email: *bffipeir@marinwater.org*

Re: Comments on the Biodiversity, Fire and Fuels Integrated Plan and Draft Environmental Impact Report

Dear Mr. Horne:

The purpose of this letter is to provide Marin Conservation League's comments on Marin Municipal Water District's (District) proposed Biodiversity, Fire and Fuels Integrated Plan (BFFIP, or Plan) and Draft Environmental Impact Report (DEIR). We appreciate the considerable work done by District staff and consultants over the past several years to reach this point. While the DEIR is a thorough document, we believe that several points require clarification and further analysis in order to fulfill the intent to use this as a Program EIR to mitigate to less than significant levels the impacts of subsequent activities. A6-1

The primary object of MCL comments is the DEIR. However, since the BFFIP itself contributes to the "Environmental Setting" for the DEIR and also constitutes the "Project Description" that is analyzed in the DEIR, we include several comments on that document as well. **Comments on both the BFFIP and DEIR that warrant a response in the Final EIR are presented in boldface type.**

1. **Environmental Setting**

BFFIP Chapter 3: Recreational use as a "threat and trend."

In describing environmental conditions on the Mount Tamalpais Watershed, the BFFIP, Chapter 3, identifies four conditions – fire and fire suppression, invasive species, forest disease, and climate change – as posing a combined threat to the health of the local ecosystem. "The combined effects of the interacting threats pose the risk of a cascade of changes that affects the entirety of the ecosystem." (DEIR, 2.4.1 Plan Need, page 2-15)

MCL believes that a fifth "threat" should be added to these four – that is, recreational uses on the watershed. Recreational visitors have grown exponentially over recent decades, from several hundred thousand annual visitors 40 years ago, to between one and three million in 2014 (DEIR Page 3.10-2). Since its founding, the District has generously provided for recreation as a social benefit, as long as the production of high quality water is not compromised. Humans also can have damaging effects on the watershed environment. The District's Road and Trail Management Plan (DEIR Page 2.6) summarizes the impacts of recreational use as follows: A6-2

> "Roads and trails can have many undesirable effects on the environment. They can increase the number of visitors and intensify human use in seldom-visited areas. They can provide migration routes for non-native invasive plants into previously un-infested areas and facilitate the spread of Sudden Oak Death syndrome. They can fragment habitats by creating migration or foraging barriers to some wildlife. They can physically remove habitat or a portion of it. . . .Furthermore, *an increase in the density and amount of human presence in previously untrammeled or seldom visited areas leads to an increase in the severity of effects and a proliferation of additional effects.*" (Emphasis added)

PHONE: 415.485.6257 EMAIL: mcl@marinconservationleague.org ADDRESS: 175 N. Redwood Dr., Ste. 135
FAX: 415.485.6259 WEB: marinconservationleague.org San Rafael, CA 94903-1977

The DEIR examines the impacts of Management Actions 20 – 27 on the recreational experience (DEIR 3.9. Noise, and 3.10, Recreation) but does not account for the contribution of recreation itself as an interacting threat in the "cascade of changes" resulting from other existing trends that are addressed in the BFFIP. This is an inadequacy of the DEIR in that it doesn't fulfill its obligation to provide a good faith effort at "full disclosure" of potential impacts as described in CEQA Guidelines Sections 15003(i) and 15151. This can be achieved in the Final EIR by doing the following:

Describe historic and ongoing impacts of recreational use on watershed resources and identify what measures will be taken in implementing the BFFIP to manage invasive plants, restore ecosystems, and minimize sedimentation along roads and trails that are exacerbated by recreation of all forms. Describe how the BFFIP Management Actions will be coordinated with the District's Road and Trail Management Plan and "Project Restore."

2. <u>**Project Description**</u>

<u>Designating a "deferred action zone" is a *de facto* "Management Action."</u>

MCL agrees that the appropriate "Project" for impact analyses are the Management Actions: MA-20 through MA-27. These all involve direct physical manipulation of vegetation and/or action that could result in a physical impact on plants or wildlife. MCL believes that an additional "action" that could have indirect environmental impacts should be added to the project and analyzed in the DEIR. The DEIR maps the extent of invasive infestations in Figures 2.3-1 – 5, showing notable concentrations around Phoenix Lake and Deer Park-Bald Hill area. Further, the DEIR presents a stunning recital of the damaging effects of broom infestations on ecosystems if left unmanaged (DEIR Page 3.3-62 ff.). The District's decision to defer active management of these large swaths of broom is due, in large part, to the handicap placed on budget resources by eliminating chemical treatment as an effective Integrated Pest Management (IPM) tool when all other management tools prove ineffective. (See further discussion under Alternatives, below). The Final EIR should address this by doing the following: A6-3

Explain in the Final EIR why deferring action is not listed as a Management Action under the Project Description. Please identify the decision by the District to defer management of this threat to the ecological health and high quality water production of the watershed as a significant indirect impact. Further, what mitigation measures could be employed to reduce the impact to less than significant?

<u>Expected outcomes</u>

The BFFIP provides a list of anticipated outcomes after five years of implementing the Plan (BFFIP Page 7-7). A somewhat comparable table in the DEIR lists the units (acres or patches) of work effort that are planned on an annual basis (BFFIP Table 2.7-1 Vegetation Management Actions). Also embedded in the text are units of planned work, such as 117 acres of "formal fuelbreak expansion," with a target to complete 50 percent, or 59 acres within five years. It is difficult to find any one table in the DEIR that provides the anticipated outcomes as shown in the BFFIP on Page 7-7. Since the BFFIP was completed almost three years ago, are these outcomes still current or should they be updated? A6-4

The Final EIR must provide a current table of anticipated outcomes listing annual targets and anticipated achievements for each of the five years under the Plan, which can be monitored and reported to the public. This table will also provide a basis for evaluating the sufficiency of future annual work plans and budgets.

The Final EIR should also explain how the District will monitor and report annually on the extent of invasive plant removal achieved, in terms of both absolute acreage and a percentage change in overall acreage of each infested community, and how much identified acreage remains. A6-5

3. Significant Impacts

Maps of existing and proposed fuelbreak system do not show impacts to special status plant species.

Since 1995, the District has completed about 900 acres of fuel load reduction – around structures and utilities, along strategic service roads, along ridgelines, and half of it within habitat. The BFFIP gives a detailed description of fuelbreak standards and maps the existing system in the BFFIP, Figures 3-11 – 13, at a smaller scale in the DEIR Figure 2.3-1, and again in DEIR Figure 2.6-1 – 4. Then, at an impossible-to-interpret scale, DEIR Figure 2.7-1 shows where new and widened fuelbreaks will be implemented under the BFFIP. At the same small scale, DEIR Figure 3.3-21 overlays planned new and existing fuelbreaks on a map of Special-status Plant species, including serpentine areas. The result is a confusing, unclear, and virtually impossible to understand jumble of information. More useful detail is shown in Figure 2.7-2, but this is only a limited example of fuelbreak expansion. This is a deficiency of the DEIR in that it is extremely difficult for the public to discern exactly what is proposed and where, and how the fuelbreaks may directly and indirectly impact special status plants. The following should be done to correct this: A6-6

Provide readable maps at a scale that shows fuelbreak construction, including both widening of existing fuelbreaks and new extensions, in relation to (overlaid on) the distribution of special status plant populations, in order to allow the public to clearly identify potential significant impacts to special status plants.

The DEIR does not identify or mitigate potentially significant impacts on common wildlife species.

The District lands are known to host at least 400 vertebrate wildlife species and to support "high levels of biodiversity." (DEIR Page 3-3-15) With the exception of the special status animals discussed in detail throughout the Biological Resources section and land birds, which have been monitored since 1996, the DEIR is woefully inadequate in its discussion of the common vertebrate species that make up the bulk of wildlife on District lands. The impact analysis presents only one impact category that offers the opportunity to identify impacts on these "other" wildlife, namely, Impact Biology-4: "The proposed plan could interfere substantially with the movement of any native resident or migratory fish or wildlife species or with established native resident or migratory wildlife corridors, or impede the use of native wildlife nursery sites."

The DEIR refers the reader to Impact Biology-1 and -2 for greater detail in analysis of impacts on wildlife, such as impacts due to tree removal, invasive species control, and use of heavy equipment and other tools to reduce fuel load. The analysis focuses exclusively on the construction and active management phase of vegetation removal work, however, and concludes that longer-term habitat alteration is largely beneficial, i.e., the resulting condition after removing vegetation will deter the future spread of wildfire. The analysis fails to identify that MA-20 and 21 (maintenance of existing and construction of new or expanded fuelbreaks) could result in long-term impacts on movement of wildlife that depend on sheltered habitat, such as chaparral. For slow moving reptiles and small mammals, for example, fuel breaks create both a barrier and an exposure hazard to movement within their accustomed habitat. In both cases, animals become easy prey for predator species or victims of human traffic. Other impacts could include the potential for permanent loss of nesting and rearing ("nursery") habitat for ground-dwelling birds. This inadequacy of the DEIR should be addressed by doing the following in the Final EIR: A6-7

Describe the significant impact of long-term habitat alteration due to the cyclical maintenance and construction of new fuelbreaks on movement corridors, and the potential loss of nesting and rearing habitat of common vertebrate species in altered habitats. Provide mitigation measures to reduce the impact to less than significant.

4. Alternatives

The "Limited Use of Herbicides" Alternative

As requested by MCL and others in commenting on the scope of the EIR, the Draft EIR presents a well-described alternative based on the addition of limited use of herbicides to the Project as proposed, where essential to achieve management objectives. Limited herbicide use would be consistent with the principles of Integrated Pest Management (IPM) and its applications highly restricted.

The environmental analysis concludes that this alternative may result in a more effective program, as herbicide use would allow more areas to be treated since it requires less equipment and workers, and less repeated work to remove weeds (DEIR Page 4-30). Although the alternative would introduce several potential effects that would not occur under the proposed Plan, none of these effects would be significant as mitigated, according to the DEIR. This alternative is supported by MMWD Policy 2.2-G in the Mount Tamalpais Watershed Management Policy (DEIR, Page 3.3-70), which states that the District will give high priority to management of exotic species, in keeping with the principles of IPM. "Methods include mechanical removal, *chemical treatment* (emphasis added), and others. . ."

Among the management actions discussed in the DEIR, MA-27 offers the opportunity to "update the Plan's vegetation management tool box and the District's IPM program as additional effective, environmentally safe, and efficient methods are identified." Under this management action, the District could reconsider chemical treatment at a future time. Such a reevaluation would also be consistent with the Plan's third goal – "Adaptive Framework for periodic review and revision of BFFIP implementation decisions in response to changing conditions and improved knowledge." For example, the recent discovery of populations of Japanese Knotweed in San Geronimo and Lagunitas Creeks since publication of the BFFIP should prompt critical reexamination of the District's current IPM toolbox. Considered the world's most invasive weed, Japanese Knotweed's spread can only be stopped with herbicides.

MA-13, although not part of the Project, calls for reviewing and updating the District's Vegetation Management toolbox to ensure that "lessons learned" are incorporated into implementation of the Plan. New techniques for managing invasive plants are constantly being developed. It is possible that new herbicides might pose less risk of exposure, or existing herbicides might prove effective at greatly reduced concentrations, particularly when used in conjunction with other, developing techniques. A6-8

The comparison of impacts of the " limited herbicides" Alternative with impacts of other alternatives and the project as Proposed, presented in DEIR Table 4.7-1, should foster informed decision making and support the opportunity for future improvements to the Biodiversity, Fire, and Fuels Integrated Plan. In order to provide a greater "full disclosure" analysis of this issue, the following should be done in the Final EIR:

Provide greater elaboration on how reconsideration of the "Limited Use of Herbicides" Alternative would fulfill the intent of:

- **MMWD Policy 2.2-G in the Mount Tamalpais Watershed Management Policy;**
- **The Plan's third Goal – "Adaptive Framework for periodic review and revision of BFFIP implementtion decisions in response to changing conditions and improved knowledge";**
- **The intent of MA-27, and**
- **The intent of MA-13.**

The Final EIR should include a table similar to 2.12-1 (DEIR Page 2-57) that compares the labor require-

ADV_GEC_MMWD_BFFIP_DEIR_MCLComments_6.19.2019

ments for each management action for each Alternative, compared with the Project as proposed.

Finally, MCL appreciates the work of staff and consultants on this Draft EIR, and we thank you for your attention to these comments. A6-9

Sincerely,

Linda J. Novy
President

Nona Dennis
Chair, Parks & Open Space Committee

ADV_GEC_MMWD_BFFIP_DEIR_MCLComments_6.19.2019

2.3.6 Letter A6: Linda Novy, Marin Conservation League

Response to Comment A6-1

The commenter notes that the purpose of the comment letter is to provide the Marin Conservation League's comments on the BFFIP. The comment is noted.

Response to Comment A6-2

The commenter states that a fifth threat, recreational uses on the Watershed, should be added to the four identified threats to the ecosystem listed in the BFFIP. The commenter suggests the DEIR describe the historic and ongoing impacts of recreational uses on Watershed resources and what BFFIP measures would be exacerbated by recreation. The comment is noted regarding Marin Conservation League's (MCL) recommendation to add a fifth threat, recreation, to the BFFIP. The District finds that the three threats as described in the BFFIP are the primary threats that drive the goals of the program, and recreation is not appropriate to add to the list. Recreation is allowable on District lands and, in fact, the Mount Tamalpais Watershed "is held in trust as a natural wildland of great biodiversity, as scenic open space, and as an area for outdoor recreation for Marin and much of the Bay Area" (District, 2019). Recreation can cause spread of invasive species and spread of invasive species is identified as a primary threat in the BFFIP already but is allowed on the Watershed. Supporting recreation is an important responsibility of the District, so it should not be listed as a threat in the BFFIP. Indirect effects of recreation, such as spread of invasive species, is accounted for in the list of threats.

Historic use and current recreation are described in Section 3.10.2 on pages 3.10-1 through 3.10-9 of the Draft EIR. Ongoing recreational activities are a part of the baseline conditions, and therefore, it would be inappropriate to assess the existing impacts of recreation as the commenter requests (CEQA Guidelines Section 15125). Baseline conditions including erosion and spread of invasive species, such as from use of trails and roads, are adequately described in various setting sections of the Draft EIR.

The EIR must only address the impacts of the proposed plan. The plan would not increase recreational usage nor involve recreational activities that could have an effect on the environment beyond existing or baseline conditions.

Response to Comment A6-3

The commenter notes to explain in the Final EIR why deferring action is not listed as a Management Action under the project description. Refer to **Master Response 2: Ecosystem and Fuels Deferred Action Zone** for an explanation as to why no impacts are associated with designation of this area as part of the BFFIP. The zoning defines the strategies that would be employed to achieve plan goals. The strategy for the Deferred Action Zone is to defer large-scale action but contain weeds where strategically possible. Maintenance activities occur in this zone under existing conditions and would continue to occur following implementation of the BFFIP. No change in the management of this zone would occur compared to existing conditions as a result of plan implementation. Any environmental effects associated with the existing weeds and diseased trees is part of the baseline conditions for the EIR (CEQA Guidelines

Section 15125). No new impacts would occur as a result of the BFFIP that need to be analyzed in the EIR.

Response to Comment A6-4

The commenter notes that the Final EIR should provide a current table of anticipated outcomes listing annual targets and anticipated achievements of the five-year plan. Refer to Response to Comment A5-3 for a discussion of the annual performance criteria for the plan. The performance criteria for each management action are presented in Table 7-2 of the March 2019 Draft BFFIP and Table 2.7-1 of the Draft EIR. These tables are current as presented in the March 2019 Draft BFFIP and Draft EIR. The BFFIP is phased gradually starting in Year 1, with full implementation in Year 5. The BFFIP does not have a specific life span; Year 5 performance criteria are assumed to occur for each subsequent year throughout the life of the BFFIP. Further details of each management action, including the long-term build-out of fuelbreaks as part of MA-21, are provided in Chapter 6 of the March 2019 Draft BFFIP and Section 2.7 of the Draft EIR.

Response to Comment A6-5

The commenter notes that the Final EIR should explain how the District will monitor and report annually on the extent of invasive plant removal in terms of acreage and percent change in infested plant communities. As discussed in Section 2.14 on page 2-59 of the Draft EIR, the BFFIP would be implemented under an adaptive management framework. Activities to be completed each year would be presented in an Annual Work Plan. After each year, an Annual Report would be prepared and presented to the District's Board of Directors. The Draft EIR already includes the information requested by the commenter.

Response to Comment A6-6

The commenter states that the DEIR should provide readable maps at a scale that shows fuelbreak construction in relation to the distribution of special-status plant populations. The commenter states that the Draft EIR does not identify special-status plant species locations in relation to new fuelbreaks. Refer to **Master Response 1: Definition and Location of New and Expanded Fuelbreaks** for a discussion of new and expanded fuelbreak locations as shown in Figures 2.6-1 through 2.6-4 and Figure 2.7-1. Refer to **Master Response 3: Special-Status Plants** for a discussion as to how the impacts from creation of new and expanded fuelbreaks on special-status plants is adequately addressed in the Draft EIR. The Draft EIR focuses on identification of rare plants during pre-work surveys and avoidance based on the species found and its life form, as defined in MM Biology-2. Some minor revisions were made to MM Biology-2, as shown in Chapter 3 of this Final EIR.

Response to Comment A6-7

The commenter states that the DEIR should describe the significant impact of long-term habitat alteration due to cyclical maintenance and construction of new fuelbreaks on movement corridors. Refer to **Master Response 4: Wildlife** for a discussion of the scope of the biological resources' analysis in accordance to CEQA and a summary of the analysis of impacts on common wildlife species and habitat according to CEQA, as adequately presented in the Draft EIR.

Response to Comment A6-8

The commenter states that the Limited Use of Herbicides Alternative is superior to the proposed plan. The commenter also requests to provide greater detail on how reconsideration of the Limited Use of Herbicides Alterative would fulfill the intent of the plans goals and policies including Policy 2.2-G, the plan's third goal, MA-27, and MA-13. The commenter's support for the Limited Use of Herbicides Alternative is noted. The BFFIP is not a true IPM program, as the commenter notes, because it does not include the judicious use of herbicides. The Limited Use of Herbicides Alternative would include the judicious use of herbicides; however, it was not the environmentally superior alternative, as explained in **Master Response 6: Limited Use of Herbicides Alternative**. The BFFIP is designed to reduce the impacts of invasive species and improve ecological health on the Watershed, even without herbicides. The plan includes criteria and goals to be presented to the District's Board annually. The adaptive management aspect of the program will allow for reassessment of methodology, as noted by the commenter, per MA-13 and other provisions.

MA-27 is described in detail on page 2-41 of the Draft EIR and includes non-herbicide experimental controls for invasive species. MA-13 is described on page 5-8 of the March 2019 Draft BFFIP and requires review and updating of management actions but does not include addition of herbicides. The BFFIP does not preclude the future use of herbicides by the District; however, any future proposals for herbicide use are explicitly not covered under the BFFIP and BFFIP EIR. Use would require additional review under CEQA and District Board approval.

According to the CEQA Guidelines the "EIR shall include sufficient information about each alternative to allow meaningful evaluation, analysis, and comparison with the proposed project." The description and analysis of an alternative does not need to be as robust as the proposed project (Section 15126.6; County of Inyo v. City of Los Angeles (1981)124 Cal.App.3d 1). A table comparing the labor requirements for each management action for the alternatives analyzed has not been prepared. This level of detail is not necessary to present the appropriate level of analysis for the impacts of the alternatives. It is noted that some reduction in labor would result from the limited use of herbicides. The Draft EIR identifies the reduced impacts associated with reduced labor and manual and mechanical methods on pages 4-27 through 4-28 and includes reduced impacts associated with Air Quality/GHG emissions, Fire Hazards, Geology and Soils, Noise, Transportation, and Energy.

Response to Comment A6-9

The comment regarding MMWD's work on the EIR is noted.

RECEIVED
JUN 19 2013
BY: ....

Comment Letter A7

Marin Audubon Society

P.O. Box 599 | Mill Valley, CA 94942-0599 | marinaudubon.org

June 19, 2019

HAND DELIVERED
Shaun Horne, Natural Resources Manager
Marin Municipal Water District
220 Nellen Avenue
Corte Madera, CA 94925

Re: Comments on the Biodiversity, Fire and Fuels Integrated Plan and Draft EIR

Dear Mr. Horne,

The Marin Audubon Society appreciates the opportunity to comment on Marin Municipal Water District's **Biodiversity, Fire and Fuels Integrated Plan** and the Draft Environmental Impact Report for the plan. The purpose of the Plan as defined in the DEIR is "to identify methods needed to reduce fuel loads and fire risks on District Lands in the plan area and to preserve and enhance existing significant biological resources." The DEIR focuses almost completely on fire protection with a few discussions of special status species. The DEIR contains important information about the watershed and actions intended to maintain it. However, scientific information necessary to support the plan, its purposes and actions, are missing. Our comments focus on wildlife and habitats, the lack of supporting data and inadequate analyses. Significant impacts are not identified and proposed mitigations are not adequate to reduce impacts to less than significant. Biodiversity is virtually ignored. A7-1

Analysis of Impacts and Mitigations Flawed

Actions are intended "to reduce firs risks and enhance biodiversity across the plan area as compared with baseline conditions." No technical and scientific data is presented to support the claimed outcomes of the actions. In fact, the approach appears to be based on assumptions, instead of science, as reflected in the statement in the Hazardous Materials section: "no modeling was performed because it was assumed that work performed and under the plan would serve to reduce fire risk...over baseline conditions." A7-2

The overriding mitigation for potentially significant impacts is that the project actions will reduce fire risk in the long term, and that somehow excuses immediate significant adverse impacts that would be caused by project actions. The assertion that the plan will "ultimately reduce fire risks across the plan areas" does not mitigate for the current adverse impacts that actions of the plan would cause, even if it were true. No scientific data is presented to support that claim. The prediction of a future fire and reduced fire risk does not mitigate immediate significant adverse impacts. The loss of vegetative habitats means the immediate loss of the current benefits the habitats provide. Those benefits include clean air, water, erosion control, temperature moderation, heat reduction, habitat for wildlife, and biodiversity. A7-3

1

A Chapter of the National Audubon Society

Fires may not ignite or destroy vegetation in the locations that are proposed to be treated (fires leave patches of vegetation). Habitats regenerate after a fire and they regenerate in a mosaic of habitats. The proposal to remove vegetation for fuel breaks and to reduce fuel loads would be ongoing, would be in locations selected by the District which may not be strategic to reduce fire, and would generate more flammable weeds- a weed monoculture. Data that supports the actions and a discussion of the individual and cumulative ongoing impacts to species and habitats of the loss of native habitats that would result from the plan need to be provided.

A7-3

Specific management actions are presented that are intended to "reduce fire risks and enhance biological diversity across the plan area as compared with baseline conditions." The plan fails to recognize that many of the management actions would cause significant adverse impacts to existing vegetative resources, habitat and wildlife, and that these impacts are not reduced to less-than-significant by proposed mitigations.

Invasion by non-native species is identified as a significant impact on the watershed, yet the plan leaves in place and huge untreated areas of the highly invasive and flammable broom. The DEIR actually presents data and analysis that supports a mitigation that could effectively rid the watershed of these flammable plants. Yet the District fails to choose this effective mitigation or alternative to address this significant impact. As a result, the broom invasions will result in increased rather than decreased fire risk and the impact must be continue to be considered significant.

A7-4

Implementing other actions may (or may not) reduce the fire risk, but they would cause adverse impacts of habitat destruction and species reduction. Modeling should be done to address the fire hazard by providing a more clear understanding and to better inform proposed actions so that both essential habitat can be protected and enhanced and fire risk reduced. The data should inform locating actions where they would be effective in reducing fire risk and also avoiding/and minimizing impacts to habitat and wildlife.

A7-5

DEIR Fails to Address Biodiversity Contrary to the Plan's Stated Goals

As stated in the DEIR, protecting biodiversity is a critical element in protecting the watershed ecosystem and ensuring the watershed is resilient to climate change. Biodiversity needs to be defined. How do you know biodiversity is being protected, if it isn't defined? Why is it important to maintain biodiversity and to have a resilient ecosystem?

A7-6

The DEIS needs to explain how the plan will ensure biological diversity is protected and restored. The proposed management actions and inactions have the potential to cause significant adverse impacts to native habitats and species. For each management action there should be a discussion of how the action will impact biodiversity, including common native species, impacts and mitigations that will ensure their survival.

The plan addresses protecting only "significant biological resources" and does not even mention wildlife, (except for NSO in an appendix). No plan that claims to address biodiversity would be complete without considering all taxa that depend on the project area, common species along with special status species, even though that may not be required by CEQA.

A7-7

All species that live in and move through the watershed and are part of the biodiversity of MMWD lands should be considered. It is important to know what they and their habitat needs are, in order to assure

2

they will have the habitats they need to survive. The Plan should identify species or species groups, habitats they depend on, and the locations and acreages where these habitats will be protected, enhance or restored. A7-7

Wildlife Impacts Not Adequately Addressed

There is no mention of wildlife in the plan, except for Northern Spotted Owl (NSO) in an appendix. This is a major flaw. Special status species are addressed in the DEIR but the discussion of non-special status is minimal and inadequate. Non-special status species are addressed in only two places in the DEIR – under wildlife page 3.3-15 and in the Invasive Species discussion (broom) on page 3.3-64 and 65. The DEIR needs to discuss potential impacts of management actions on all wildlife. The interconnectedness of plants, animals and water (in other words how an ecosystem functions), should be explained. A7-8

The DEIR recognizes that the District Lands are important for maintaining a diverse bird community but there is no further discussion. The discussion should not end here. Biodiversity dictates that the District ensure habitat for all species, and the declining species in particular. Please discuss how the Plan will avoid further declines in wildlife populations caused by fuel reduction and failure to remove invasive species? A7-9

Common Species: The discussion on page 3.3-15 Common Wildlife states that at least 400 species of vertebrate animals are found on District lands, and that these lands are included in UNESCO's Biosphere Reserve system because they support "high levels of biodiversity in a large-scale landscape that is protected from development." Provide more information on the Point Blue 2014 monitoring of common species, one of the few references to non-special status species in the DEIR. Point Blue analyzed trends and abundance for 44 species. How were they chosen, and what habitat types do they require for survival? The Point Blue study reports: that Scrub Jay and California Towhee are significantly declining while Pileated Woodpecker and Stellar Jay are suffering marginal declines. What measures have or will be taken to reverse the declines. A7-10

The other discussion (pages 3.3-64 and 5) under 3.3. Invasive Species reports from studies on the impact of broom on GGNRA lands. Examples of native wildlife that may be adversely affected: Red-tailed and Red-shouldered Hawk, American Kestrel, Northern Harrier, California Quail, many species of sparrow owls, finches, Juncos, Bewick's and Pacific Wrens, Swainson's and Hermit Thrushes, Orange-crowned Warbler, Allen's and Anna's Hummingbird, NSO, Coopers Hawk, numerous native reptiles and mammals, shrews, chipmunks, voles, deer mice, brush rabbits. Destruction of habitat for these many species should be identified as a significant impact and effective mitigation should be presented to reduce the specific impacts of both leaving large areas of broom and, at the same time, removing understory habitats for fuel breaks and to reduce fuel loads. Both of these actions will remove native habitat and should be considered significant impacts. Mitigation to assure habitat for these, and the many other species that would likely be impacted, as well as for increased fire risk, must be presented. A7-11

An impact analysis should be provided that addresses impacts to all of the ground dwelling species and species that depend for some part of their life cycles on the habitats that will be impacted by District actions or inactions. This would include review the nesting and non-nesting habitat needs for each of species or representative species and evaluate whether and how the management activities or in activities that are planned could adversely impact these species. Of particular concern are birds and mammals that forage and nest on the ground (e.g. quail, towhee, sparrows) and those that depend for A7-12

3

some portion of their life cycle (hunt, forage, nest, rest, cover) on types of habitats the plan will adversely impact. How will the district ensure there are no habitat reductions for the declining species, in particular the special status and declining species noted above, and ground dwelling species? The mitigation analysis should ensure habitat types sufficient to support wildlife populations do not decline. A7-12

There is no population trend analysis for mammals, reptiles or amphibians. These taxa are essential parts of the ecosystem and also would be adversely impacted by the loss of understory habitat. There should be surveys and trend analyses. The discussion simply states "Given the extent of observations by District staff and others – their occurrence is well documented." Present what the District knows about their occurrence, population status and trends? A7-13

Special Status Species: The DEIR recognizes that many of the project actions would have significant adverse impacts of special status wildlife species, but that the proposed mitigation measures would reduce the impacts to less than significant. We find many of the mitigations lacking. A7-14

Twenty special status bird species are listed as nesting on District lands but there is no discussion about impacts to these species, except Northern Spotted Owl. How the habitats for these species will be impacted by the plan must be discussed.

Northern Spotted Owl (NSO) – The wildlife species receives most attention in the plan and in the DEIR is the NSO. The District, in partnership with the MCOSD, contract with Pt. Blue Conservation Science to conduct surveys for NSO annually since 1999. How much of the NSO activity center areas would be subject to understory clearing for fuel reduction or fuelbreaks construction? How much of that is native vegetation?

Of the 117 acres of new or widened fuel breaks and fuel reduction areas 58 are within 0.25 miles of a NSO nesting, foraging habitat. Protections include avoiding work near nests during nesting season. The DEIR acknowledges that fuel break creation could impact NSO and Dusky-footed Woodrat habitats through removal and thinning of vegetation. Noise could also impact NSO.

According to the discussion, vegetation removal would promote the growth of a diversity of vegetation, which supports a wider prey base page 3-3.3-78 and would favor the NSO. This is a questionable statement as there is no evidence diversity or a wider prey base for the NSP, who primarily feed on woodrats, would result from the BFFIR activities. Vegetation removal for fuelbreaks and fuel reduction could very well result in more broom and reduce diversity. A7-15

The mitigation measures address impacts during construction. The longer-term impacts of removing vegetative habitats must also be addressed.

The DEIR Justifies fuel breaks in NSO habitat by the observation that the watershed has one of the most "prolific northern spotted owl populations in the State." This is an unsubstantiated connection. NSO populations in other parts of the state are subject to significant pressures from logging which ours do not have to contend with. Further, it is not known whether fuel break activities are adversely affecting NSO by destroying habitat for the NSO and their prey Dusky-footed Woodrat. If the District has done studies and has data to support this claim, it should be presented.

Mitigation Measures for NSO

MM8 Avoidance during Nesting Season Alternative 1. Explain why, mowing, burning, or mechanical A7-16

4

removal of vegetation would need to occur during the NSO nesting season. The nesting season dates should be modified to extend from February 1 through July 31. Why can't nest trees and buffers be avoided during this period? We agree that if a nest is found, work should be delayed until after Sept 1. However, just as important are the potential longer-term impacts of the vegetation removal be done in this particular location? Discuss the survey date for nests near fuel breaks, fuel reduction zones and NSO nest sites and Core habitats?

Alternative 2 should be dropped. It is based on a calculation using USFWS Guidance on Auditory and Visual Disturbance. This document is based on and was developed regarding NSO in the lumbering areas of northwest. The Marin County NSO population is different in many ways. A7-16

MM14 Avoidance of Nesting season and Habitat. This addresses vegetation removal in NSO core areas. This mitigation actually is not avoidance. It requires that for projects within 0.25 mile of NSO activity center surveys be conducted under the guidance of a qualified biologist and that GIS data be consulted. Why it is not recommended to consult with Pt. Blue data which is being collected for the District and the OSD annually since 1999? The Pt. Blue data is current. The survey data from 2017 and 2018 are available and the information in the DEIR should be updated and they be separated and only the population on MMWD lands should be referenced here. The population numbers appear to include lands own by both jurisdictions. A7-17

Analyze what areas would be avoided and compare with the planned fuel reduction and fuelbreak areas enable the significance of avoiding these areas to be assessed. Perhaps it would not be many acres. Actual avoidance of the work so that direct nesting impacts and long term loss of habitat is the alternative that should be chosen. A7-18

For areas within NSO Core habitat - Management Activity 1 calls for retaining a mix of disturbed and undisturbed habitat. This is too vague to ensure the action would be effective. As noted in the discussion, maintaining a healthy prey base is critical for the owl survival. What does a mix of habitat types mean, how many acres and where? Does the District know where the woodrat nests are? What habitat mix would be adequate? Further, it is not clear why any NSO activity center habitats need to be disturbed at all. Following the CEQA preferred mitigation of avoidance, provide biological support to and demonstrate that vegetation removal is needed in NSO activity center areas. A7-19

We completely agree with the mandate in #2 woodrat nests "shall" be avoided, under any vegetation management activity, but clearing around nest structures is a concern. It seems to be assumed that if Dusky-footed woodrat houses are avoided there would be no impact at least due to diminished prey base. Vegetation clearing around or near them. May leave woodrats more vulnerable to predation and/or less food.

Discuss the importance of vegetation within woodrat foraging range as it pertains to maintaining their populations. Would clearing vegetation at a distance around the nests reduce their food source and/or render them more vulnerable to predation, so as to indirectly effect NSO food source? Could their population decline because there is less vegetation for them to eat, or because they are more exposed to predators? How the woodrat houses would be identified? Would pre-project surveys of woodrats required? Are there any surveys being conducted currently? It seems important to know where they are located because the sticks that conceal their nests are targeted for removal by fuel load reduction actions. A7-20

5

It should also be noted, that NSO use habitat beyond the ¼ mile boundary for hunting and disbursal areas for young when they leave the nests. A7-20

Mitigations for Other Special Status Species

Mitigations proposed for special status species are basically the same for most species: do surveys, avoid work during the nesting/breeding season if the species is nesting.

For Badgers the mitigation includes marking dens and, if occupied during breeding season, avoid work within 100 feet of dens. There is allowance for badger relocation with a plan submitted to the CDFW. Why is relocation proposed at all? Has this been done before, where and with what success? Where would they be relocated to? We recommend deleting the provision to allow intrusion into the 100 foot buffer. A7-21

Western Pond turtle mitigation includes either rescheduling if eggs are present, avoiding work within 100 feet." Western Pond turtles burrow in the mud so turtles could be impacted directly or work done is done with the pond habitat during winter. Discuss this further. A7-22

Red Legged Frog and Yellow Legged Frog – For Yellow Legged frogs work will be rescheduled until after eggs hatch but could be relocated; for Red-legged Frogs area should be avoided until after eggs hatch. Why are the requirements different for these two species? Even though there is only one citing of RLF, We recommend the more stringent requirement be applied to both species. There may be more. A7-23

Giant salamander mitigation also allows for relocation. Why is this drastic measure proposed for this special also? A7-24

For the many species of bats, the mitigation provides for various conditions to removing active maternity tree after breeding and methods to remove tree used as a roost. Explain conditions that would be so urgent at to remove maternal trees or roost trees? We strongly recommend they not be removed at all. A7-25

Habitat impacts: The recommendation is to avoid wetlands – except for seasonal wetlands where work should only occur during dry season. Work that could adversely impact any wetlands, including seasonal wetlands, in the short or long-term should be avoided. Impacts could result from filling, draining, destroying or modifying wetland vegetation. All of these activities in any wetland need permits from regulatory agencies. Permitting agencies include the RWQCB and possibly Corps of Engineers. A7-26

Habitat Restoration: Only three habitat restoration projects are identified, as discussed at MA 26: Porter Meadows, Sky Oaks Meadow and Incision Island. The District should commit now to preparing an EIR or Mitigated Negative Dec on these projects for which specific actions have not yet been identified. The wildlife and plant species each of these restorations would benefit should be stated. A7-27

Management Actions Not Adequately Described or Mitigated

Nineteen of the 27 management actions have to do with administrative actions. How many of the 19 actions not reviewed are new activities and how many are continuations of ongoing planning, monitoring, inventories and collaboration the District has been doing for years? A7-28

Proposed management actions are: maintaining fuel breaks, creation of new or expanded fire breaks, removing SOD, fuel reduction zones, prescribed burns and removal of invasive vegetation.

6

Fuel Breaks

Show all of the new or expanded fuel breaks on a figure. Figures 2.7 1-2 only show examples.

The discussion each new or expanded fuel break should describe whether the expanded area is vegetated with native or invasive plant species? What species of wildlife depend on the habitats where vegetation will be removed for the expansion? Discuss the habitat impacts for removing native vegetation. What is the acreage for new fuel break areas?

Describe the criteria used to decide which fuel breaks to expand. What is the primary reason for locating fuel breaks where they are? Is it for access or to fight fires? Are fuelbreaks are intended to reduce fire risk, slow or stop fires? Describe the intensity of fire the fuel breaks will assist in reducing.

How were the fuel break locations chosen? The choice of locations seems arbitrary. Include figures showing all of the expanded fuel breaks, not just representative areas. A7-29

The District currently has 450 acres of fuel breaks with a goal of 567 acres. Maintenance of fuel breaks is planned to occur every five years. This means vegetation, most likely broom, will be growing for five years before it is removed again, and that the District has created conditions where a flammable weed will invade. This is contradictory to the purposes of the plan and must be identified as a significant impact. A7-30

Fuel Load Reduction Areas

There is no discussion, criteria or figures showing where these areas would be located. How will areas where fuel would be reduced be chosen and what criteria is used? These areas are a particular concern because there is so little information about them.

Describe how the fuel reduction will contribute to forest health? Describe the vegetative habitats that would be removed? Would they be: native, invasive, understory shrubs, Forbes, downed trees, sticks, leaves? Describe the value of shrubs, and other material that would be removed to the ecology of the forest, to soils, plants and wildlife? Provide a response in this context to the identical impacts discussed on pages 3.3.-64 and 64 in relation to invasive broom.

Discuss whether they would be in strategic locations that would reduce fire hazard? Reduce disease? How would the removal reduce disease? How would it be assured that Dusky Footed woodrat houses would not be destroyed as is committed to on page??? A7-31

Tree Removal Actions

What is the current status of SOS in the watershed? Is it increasing, decreasing, static? Is there any research on the effect of removing SOD infected trees from a forest?

Is the intent to remove all or a portion of the trees infected with *phytophtera ramorum* (SOD)? If a portion, what proportion would that be?

Describe habitat and wildlife impacts of the spread of SOD?

Discuss the ecological value of leaving some dead and dying trees in a forest. How would the massive removal of dead trees impact biodiversity, the forest ecosystem and improve resilience? How do wildlife use them? Describe the benefits, if any, of dead and dying trees to insects, foraging and nesting birds and mammals. A7-32

7

Discuss the "disease resistant" species that would be used to replace the SOD trees? Are these species native to the area of the watershed and the habitat type where they would be planted? If so, why are they not growing there now, or are they?

How would different replacement tree species change the natural conditions of the habitat? How would wildlife populations be impacted by the new species? Would the new replacement species provide habitat for the existing wildlife? Would they serve the same functions in the forest ecosystem as the trees that have died of SOD?

How are decisions made about trees to be removed and retained?

Where are the "more degraded forest types" that have transitioned as a result of SOD? What forest types are they? Describe the biological/habitat degradation that has occurred? The discussion addresses tanoak infestation affecting aesthetics and recreation. How would wildlife be impacted? A7-32

References to treatment for SOD are made in a few places. The only discussion of "treatment" we can find is of removing infected trees. There are treatments that have been known to cure or halt SOD infections. Realizing that it would not be affordable, or perhaps even desirable, to treat all potentially infected trees in the watershed, has or would the District considered trying this chemical treatment?

MA-23 Improve Conifer and Mixed Hardwood Stand Structure in the Ecosystem Restoration Zone. This action would reduce slash and brush density in conifer and mixed hardwood forest to improve overall forest function by treating areas with heavy equipment...up to 20 acres. How are the areas chosen for this action? The District has actually been doing this for some years. What are the results of these actions that would justify their continuing? What species have regenerated? Any invasive species? What pre- and post-project surveys have been done to document the success of this approach and what have been the impacts on wildlife?

Prescribed Burns

The most biologically sound and comprehensive discussion on page 4-21 of the DEIR points out that fire is necessary for the proper functioning of the forest ecosystem. Its mosaic patterns are natural and help create a heterogeneous forest of different age classes, successional stages and species diversity. Fire recycles nutrients, prepares the seedbed for plants to regenerate, facilitates germination in some places, opens up the forest for pioneer species to establish, affects wildlife innumerous ways, and influences pest populations and disease development. "With such clear and numerous benefits, why is broadcast or prescribed burning a more important feature of the District's plan? A7-33

Pile burns, in which forest material is put in piles and burned, are a feature of the plan provide few if any of the benefits of burning a system in place. Flaming also appears to have limited ecological value because it appears they are proposed for use in only in grassland areas.

Why are these procedures favored when they have such limited benefits? Discuss the possible expansion of the use of prescribed burns as part of the plan.

Invasive Plant Removal

Invasive species removal is an essential action to protect habitats, wildlife and biodiversity. Weeds, particularly with three broom species and other species, are expanding rapidly. By the DEIR's admission, A7-34

8

the District is not keeping up with the challenge and that is resulting in failure to meet one of the major goals of protecting biodiversity, is risking natural ecosystems and increasing fire risk.

The DEIR reports (p. 109) that the District does not have the capacity to remove all of the broom with current resources. More than 1,500 acres of District land are infested with priority invasive species. In 2013, the District calculated that broom alone was spreading at a rate of 56 acres a year. Until 2005, the District was successful in reducing non-native trees using a combination of techniques including herbicides and completely eliminating eucalyptus and acacia. Table 3.3-7 shows substantial expansion of broom in the five year period from 2009 and 2014. It can reasonably be assumed that at the least the same expansion rate if not more has occurred over the last 5 year period during which the District has not used herbicides. Explain how the proposed BFFIP will correct this failure. A7-34

It is noted that glyphosate has been identified as "probably carcinogenic to humans". The DEIR should include information on the conditions under which glyphosate use presents a health risk to humans. It is our understanding that is it primarily ingesting the chemical in food, or when individuals are exposed for significant periods of time such as spraying crops. Is there a scientifically verified health risk for people who are just walking nearby, not eating food treated with the chemical or spending long periods breathing in vapors? A7-35

Describe the District's procedures for using glyphosate on broom when it was successfully treating, and compare them to conditions that pose a health risk.

Describe how Bay Area open space districts, other water districts and land managers use herbicides. Is the use of herbicides part of their broom control program? Specifically, what do the Francisco Rec and Park, East Bay Regional Parks, Marin County OSD use? What protocol do those other entities use? What is their success eliminating broom under this approach? Are there other herbicides, besides glyphosate, they use to kill broom? A7-36

Maps 2.3-1 through 5 show the areas of broom on watershed lands (1400 acres), particularly in the Deer Park (135.6 acres), Phoenix Lake, Bon Tempe, and Lagunitas Lake areas and along roads. The District plans no action in these large broom-infested "compromised" areas. These untreated areas will result in increased expansion of the highly flammable broom, increased loss of habitat, increased fire risk, as well as increased liability should a fire erupt at one of these compromised areas. The District's broom contributes to or causes a condition of extreme risk fire. For these reasons it should identified as a significant impact and effective mitigation measures recommended. The recommendation should be based on biology, not politics. A7-37

Discuss the District's potential liability should a fire erupt in the Deer Park area that is overrun with broom which will continue to expand over time. The DEIR reports that broom also occurs on adjacent properties at Deer Park. What is the potential for efforts coordinated with these adjacent landowners, to remove the broom? A7-38

Action MA-27 Conduct Experimental and Trials to Identify Suitable Methods for Control of Invasives. Is this action precluded from experimenting with using herbicides or could herbicides be part of it? How would an experimental method be defined? Would a method being used elsewhere be considered experimental if it has not been used by the District? A7-39

9

Other Vegetation Management Alternatives Description and Analyses Flawed

Our review of the proposed alternatives indicates that some of the analyses are flawed. All accept the No Burn alternative and No Project alternatives have benefits and have the potential to "avoid or substantially lessen any of the significant effects of the project." Significant effects that would be avoided include continued or increased expansion of broom and increased risk of fire in locations where broom is expanding. A7-40

Refocused Effort Alternative - This alternative would refocus efforts away from forests, grassland and oak woodland, reduce broadcast burning, and refocus on additional broom removal in areas around "compromised" fuelbreaks which are not currently proposed for removal.

We don't understand some of the overview analyses in the stated conclusions. Please explain: Why the long term impacts on habitat are likely to be greater under this alternative? Why continuous removal of broom is considered a negative for this alternative? Wouldn't continuous removal of broom be required, although on a smaller land area, and be equally negative or positive under the preferred alternative? The only alternative in which ongoing need for broom removal would be significantly reduced is the Herbicide alternative. A7-41

"More areas (would) be treated year after year" with this alternative as compared to the preferred alternative but in the long run the infestations and consequently the need for treatment would be reduced. With the preferred alternative fewer acres treated year after year but the need would go on for a longer time and the current broom infested areas that are not being treated would continue to expand. Removing the larger areas and restoring other native species in these areas would significantly reduce the need to remove broom in the long term.

We agree that impacts on birds and other tree dependent species would be substantially less with this alternative. Yet at the same time the analysis finds that the long term impacts to forests would continue because "forests diseases would continue to spread." Again, any preferred forest treatment preferably restoration, could be resumed after the broom is removed.

No Broadcast Burn Alternative - We have no comments on this alternative except that we believe it has no supportable benefits to the watershed ecosystem and should not be considered. A7-42

Limited use of Herbicides Alternative - Under this alternative three conventional herbicides would be allowed only as an initial control tool to stop expansion, in addition to all of the other removal actions. Use would be reduced as soon as it is feasible to use other methods. A7-43

Have other land managers (SFRP, MCOSD, and EBRP) herbicides to stop expansion and then moved to other procedures such as manual or mechanical to get rid of broom? A7-44

Are there any other herbicides, besides the three identified in the DEIR, that have been used successfully to get rid of broom? If so, they should be allowed to be used also. A7-45

A list of 13 restrictions that would ensure protection of water quality, the public and the environment is presented. It is a thoughtful list but we have a few comments on the list: A7-46

#8 How far in advance would areas to be treated be posted to give people advanced notice to not visit if they so choose should be stated? People plenty of time to plan to avoid the areas.

10

#13 100 foot buffer is identified as the distance to not come closer to a stream. Distance from a seasonal wetland should not be determined on a case-by-case basis. The distance from any wetland should be the same as from streams. A7-47

#14 Would restrict herbicide use to the dry season between June 1 and Sept 15. This does not take nesting season into consideration. While it is unlikely there would be nests in broom stands, there should be surveys in the vicinity of the treated area and, if nests are found, treatment should be delayed until after nesting season. Limited use of herbicides would not only reduce the need for mechanical and manual removal but would reduce the amount of broom in the watershed thereby allowing for habitat restoration. A7-48

We agree that allowing for limited use of herbicides as part of an IPM program meets all the goals and objectives of the program and would be "a more effective program for reducing fire hazards and improve biodiversity and habitat across the plan area." We disagree that it would bring "additional risks to human health, habitat and water quality." These potential impacts would be reduced to less than significant by the proposed mitigation measures including very limited use, timing, application restrictions and others above that we recommend. A7-49

The reason given for this Alternative not being chosen as the preferred alternative is that it received "minimal community acceptance." We strongly disagree with this characterization. While there was a large group opposed to any use of herbicides, there was also a large group of individuals and organizations that advocated for protecting the integrity and health of the watershed and supported limited use of herbicides to remove the highly aggressive weeds that are outcompeting the District, adversely impacting the ecosystem, and increasing the risk of fire. Even though District decisions have been made on a perception of public input, DEIR recommendations should be based on sound biological and technical and science. A7-50

Conclusion

In conclusion, we recommend that the DEIR revise the significant impact discussions and provide adequate information about recommended actions and their impacts on wildlife and biodiversity, analyses of immediate impacts that recognize the loss of native habitat and the threat to fire and habitat of not deciding on retaining the large areas of highly invasive broom. Significant impacts and impacts should be more accurately defined to ensure effective mitigation. A7-51

Thank you for considering our input.

Sincerely,

Barbara Salzman, Co-chair
Conservation Committee

Phil Peterson, Co-chair
Conservation Committee

11

2.3.7 Letter A7: Barbara Salzman, Marin Audubon Society

Response to Comment A7-1

The commenter identifies the purpose of the plan and states that the plan is focused almost completely on fire protection with few discussions of special-status species. The commenter's opinion regarding the adequacy of the analysis and mitigation is noted. The Draft EIR focuses on the environmental impacts of implementation of the BFFIP and not just on fire protection. Section 3.3 Biological Resources of the Draft EIR dedicates 140 pages to the baseline and assessment of plan impacts on special-status species, wildlife movement, and habitats including sensitive and riparian habitats. The Draft EIR identified the following:

- 20 sensitive plant communities found in the plan area (see Table 3.3-4 on pages 3.3-26 to 3.3-28 of the Draft EIR);
- 43 sensitive plants known to occur or with potential to occur in the plan area (see Table 3.3-5 on pages 3.3-30 to 3.3-37 of the Draft EIR); and
- 46 special status wildlife species known to occur or with potential to occur in the plan area (see Table 3.3-6 on page 3.3-44 to 3.3-52 of the Draft EIR).

The Draft EIR also identifies 17 "Biology" mitigation measures to protect sensitive communities, plants, and wildlife species.

The analyses are supported by substantial evidence in the form of previously published field studies, data from natural resource databases, habitat mapping performed via fieldwork and lidar imagery, professional journal articles, GIS datasets from organizations such as Point Blue and from CDFW, the input and opinion of professional biologists, and many other sources. References cited in and supporting the biological analysis are presented on pages 3.3-134 to 3.3-139 of the Draft EIR.

The project objectives of the BFFIP are found in Section 2.4 on pages 2.2-12 to 2.2-17 of the Draft EIR and are presented in Table 2.6-1 on page 2.2-17 of the Draft EIR. The objectives and goals of the plan are to minimize the risks of wildfire, to preserve and enhance existing significant biological resources, and to provide an adaptive framework for the periodic review and revision of BFFIP implementation decision in response to changing conditions and improved knowledge.

Response to Comment A7-2

The commenter states that no technical and scientific data is presented to support the claimed outcomes of the plan to reduce fire risks and increase biodiversity. The comment is unclear in terms of the scientific information requested to support the actions. The analyses and conclusions in the Draft EIR are supported by substantial evidence in the form of previously published field studies, data from natural resource databases, habitat mapping performed via fieldwork and lidar imagery, professional journal articles, other reports issued by agencies on relevant topics, GIS datasets from organizations such as Point Blue and from CDFW, the input and opinion of professional biologists, and many other sources. The actions are proposed as part of the program. Outcomes will be assessed against performance criteria on an annual basis to evaluate the effectiveness of the program. Modifications to the methodologies used would be implemented under the principles of adaptive management, in accordance with MA-13 and as described in Section 2.14 on page 2-59 of the Draft EIR. The outcome of the actions will be assessed against empirical evidence collected each year.

The commenter states that the overriding mitigation for potentially significant impacts is that the project actions will reduce fire risks in the long term, and the commenter cites that no modeling was performed for fire spread, demonstrating the commenter's opinion that the plan is solely based on assumptions that are not supported. The comment also seems to raise a question regarding supporting information on the efficacy of the proposed types of fuel treatments in reducing wildfire risks. The BFFIP includes many actions that are scientifically proven to reduce fire risks, including control of invasive species. The characteristics that influence flammability of vegetation are identified in Section 3.7 Hazardous Materials and Fire Hazards of the Draft EIR. Fire is identified as one of the key threats on District lands. Key factors present on District lands that relate to increased risk of catastrophic fire are decades of fire suppression and high levels of fuel, particularly from the thousands of trees that have been killed by SOD since 1995 and the spread of invasive species. The 1995 VMP was prepared decades ago and does not address many of the current threats. As noted in Chapter 3 Threats, Trends, and Strategies of the March 2019 Draft BFFIP, the general consensus among climate scientists is that global climate change will result in more frequent and larger fires in California (OEHHA and CalEPA, 2008). One of the three goals of the BFFIP is to reduce catastrophic wildfire risk. Several management actions would reduce fuel loads and fire risk, such as MA-20, MA-21, and MA-23.

The greatest reductions in wildfire severity by vegetation management techniques have been found to come from vegetation thinning that changes canopy fuels, favors larger trees, and are followed by fuel load removal. Reducing surface fuels, particularly through prescribed burning, is well supported by science for moderating potential wildfire behavior for up to a decade (Martinson & Omi, 2013). Research has found that thinning of vegetation and broadcast burns in tandem have been found to reduce the severity of wildfires (Kalies & Kent, 2016). The BFFIP was prepared using knowledge collected from decades of vegetation management on District lands and the ever-evolving literature on land-management treatments.

Section 3.7 Hazardous Materials and Fire Hazards of the Draft EIR provides a robust wildland fire-risk analysis. Each tool and technique is analyzed to determine whether risk of fire could increase during implementation. For example, pile or broadcast burns could escape and spread, resulting in an uncontrolled fire. This increase in fire risk during a pile or broadcast burn is identified as a potentially significant impact. Mitigation and compliance with applicable regulations would reduce this risk of a prescribed burn becoming uncontrolled. Extensive literature supports the use of fuel and vegetation management to reduce risk of catastrophic wildfire. Appendix C of the BFFIP includes a reference list of existing MMWD data and research. The BFFIP, Chapter 3.4, discusses the 2006 review of fuelbreaks and the modeling of fuelbreak placement for maximum benefit. A background report entitled Marin Municipal Water District Vegetation Management Plan Update, Background Report No. 6 Fire Hazard

Management (Leonard Charles Associates and Wildand Resource Management, 2008), has been added to the BFFIP and Appendix C of the BFFIP to support the discussion of fire-hazard modeling used to determine fuelbreak locations. The fuelbreaks proposed in the BFFIP align with those identified in 2006. While conditions have changed, these locations along roads and near the WUI remain the most effective locations for fuelbreak expansion.

The request to conduct wildfire modeling to determine the overall risk reduction following BFFIP implementation is noted. Such modeling is possible but is not considered necessary to assess hazards associated with implementation of the BFFIP. Any environmental effects resulting from the CALFIRE Fire Hazard rating of "high" to "very high" are existing effects and considered part of the baseline conditions for the EIR (CEQA Guidelines Section 15125). The purpose of the BFFIP is to identify methods needed to reduce fuel loads and fire risks. Therefore, the statement quoted by the commenter that "…work performed under the plan would serve to reduce fire risks…" is accurate and reliable.

The commenter is incorrect that the Draft EIR does not mitigate immediate significant adverse effects. Section 3.3 Biological Resources includes 17 mitigation measures to reduce significant adverse effects of the plan, including on a range of listed plant and wildlife species, avian species, and habitats. Contrary to the commenter's assertion, the fact that the BFFIP will "ultimately reduce fire risk across the plan areas" is not intended as "overriding mitigation." Without further specificity in the comment, no additional response is merited.

Response to Comment A7-3

The commenter states that the loss of vegetative habitats and removal of vegetation means the immediate loss of the current benefits the habitats provide and questions that the locations of vegetation removal may not be strategic to reduce fire and would generate more weeds. The commenter requests more data to support the actions and the individual and cumulative impacts of the BFFIP.

Clearing of large areas of vegetation is not proposed as part of the BFFIP, and significant alteration of habitat, as the commenter suggests, would not occur under the BFFIP actions, including creation of 117 acres of new fuelbreaks. As discussed in the **Master Response 1: Definition and Location of New and Expanded Fuelbreaks** and in the Draft EIR, a fuelbreak is not a denuded area without trees or other vegetative cover. While the work alters the habitats, it does not destroy or completely remove the habitat such that habitat is lost. The total acreage of fuelbreaks is approximately 4 percent of the BFFIP program area. Expansion of fuelbreaks could result in the spread of invasive species, as the commenter notes (see Response to Comment A3-7). The purpose of the fuelbreak is to provide an area of defense and improve containment in the event of a wildfire. Once a fuelbreak is created, it would be maintained per MA-20, including to reduce invasive species in the new fuelbreak. As stated under Response to Comment A7-2, the commenter's request for data is unclear. The analyses and conclusions in the Draft EIR related to potential impacts to wildlife habitat are supported by substantial evidence in the form of previously published field studies, data from natural resource databases, habitat mapping performed via fieldwork and lidar imagery, professional journal articles, GIS datasets from organizations such as Point Blue and from CDFW, the input and opinion of professional biologists, and many other sources.

The individual impacts of implementation of the plan on species and native habitats are addressed in Section 3.3 Biological Resources of the Draft EIR. Refer to **Master Response 4: Wildlife** for a discussion of the scope of the biological resources analysis in accordance with CEQA, including habitat impacts. All potentially significant impacts to species and native habitats would be reduced to less than significant with mitigation. The cumulative impacts are addressed in Section 5.1.4.3 on pages 5-20 through 5-23 of the Draft EIR and would also be less than significant. The commenter does not provide enough specificity as to what aspects of the individual and cumulative impact analyses and mitigations they find deficient to enable the District to provide a more detailed response.

Response to Comment A7-4

The commenter states that the plan leaves in place huge untreated areas of invasive and flammable broom. The commenter appears to be referring to the Ecosystem and Fuels Deferred Action Zone and is therefore referred to **Master Response 2: Ecosystem and Fuels Deferred Action Zone** (and Responses to Comments A5-7 and A6-3). Maintenance activities occur in this zone under existing conditions and would continue to occur following implementation of the BFFIP. No change in the management of this zone would occur as compared to existing conditions. Any environmental effects associated with existing and ongoing activities in this zone to treat existing weeds and diseased trees are part of the baseline conditions for the EIR (CEQA Guidelines Section 15125). No new impacts would occur as a result of the BFFIP that need to be analyzed in the EIR.

The commenter also states that "the DEIR actually presents data and analysis that supports a mitigation that could effectively rid the watershed of these flammable plants." The commenter appears to be referring to the Limited Use of Herbicides Alternative. Refer to **Master Response 6: Limited Use of Herbicides Alternative** for a discussion of the herbicide alternative. The BFFIP is designed to reduce the impacts of invasive species, such as broom, and improve ecological health on the watershed, even without herbicides, through weed removal using hand and mechanical methods. As previously stated in Response to Comment A7-2, actions described under the plan reduce fire risks as compared with the existing condition. Broom invasions would not increase under the plan, as the commenter states, because the plan includes manual and mechanical methods of treating broom to a greater extent than is currently implemented.

Response to Comment A7-5

The commenter states that modeling should be performed to address fire hazards and to better inform proposed actions so that habitat can be protected, and fire risk reduced. Refer to Response to Comment A7-2 for a detailed response regarding the fire hazard analysis. The BFFIP, in Chapter 3, Section 3.4, discusses the 2006 review of fuelbreaks and the modeling of fuelbreak placement for maximum benefit. A background report entitled Marin Municipal Water District Vegetation Management Plan Update, Background Report No. 6 Fire Hazard Management (Leonard Charles Associates and Wildand Resource Management, 2008), has been added to the BFFIP and Appendix C of the BFFIP to support the discussion of fire-hazard modeling used to determine fuelbreak locations, reason for their locations, and the basis for their section. The fuelbreaks proposed in the BFFIP align with those identified in 2006. While conditions have changed, the fuelbreak locations along roads and near the WUI remain the most effective locations for fuelbreak expansion in order to allow access to fight fires and to slow spread of fires.

The commenter also notes that the management actions should be chosen to protect habitats. Habitat protection is addressed in the BFFIP. Section 3.3 Biological Resources of the Draft EIR provides a robust analysis of direct impacts on wildlife and indirect impacts on wildlife from changes to habitat. Appropriate mitigation measures are prepared to reduce the impacts on wildlife species, as stated under Response to Comment A7-3. All potentially significant impacts to habitat and biological resources would be reduced to less than significant with mitigation.

Response to Comment A7-6

The commenter states that the plan does not define or discuss biodiversity and that wildlife are not mentioned in the plan. Both statements are inaccurate. Chapter 1 of the March 2019 Draft BFFIP describes the importance of biodiversity in the Watershed and the need for a resilient ecosystem, including in the inset on page 1-3 of the March 2019 Draft BFFIP, which states,

> Biological diversity is often used as an indicator of ecosystem resilience and environmental goods and services such as clean air and water. On Mount Tamalpais, biodiversity is at risk from the expansion of non-native invasive species, climate change, and Sudden Oak Death and other diseases.

For further clarification, a definition of "biodiversity" has been added to Chapter 1 of the BFFIP, page 1-1, as follows:

> Biodiversity is the short form of "biological" and "diversity." The term biodiversity is defined as all the variety of life that can be found in an area, including communities, habitats, and species.

Chapter 2 of the March 2019 Draft BFFIP elaborates on the existing setting, including identification of the diversity found on the watershed, as stated on page 2-1 of the March 2019 Draft BFFIP,

> The Watershed supports a rich variety of vegetation communities, ranging from grasslands to chaparral, oak woodland, and redwood forests. Vegetative communities provide habitat for a wide range of wildlife, including a number of plants and animals with regulatory protections. The Watershed supports approximately 40 special-status plant species within 88 distinct plant assemblages as defined by the National Vegetation Classification System (CNPS 2014).

Section 2.4.2 of the March 2019 Draft BFFIP provides further background information on the biological resources found on District lands. The first paragraph under Section 2.4.2 on page 2-16 of the March 2019 Draft BFFIP states,

> The total number of species within District lands is unknown, but it includes over 1,000 species of vascular plants, over 200 species of lichens, and at least 400 species of vertebrate animals. Many more species of fungi, non-vascular plants, and invertebrates such as insects and other arthropods occur within District lands. District lands are included within the Golden Gate Biosphere Reserve, created by UNESCO in 1988, because they support high levels of biodiversity in a large-scale landscape that is protected from development (District 2012a).

The commenter also states that the impact analysis should address how the actions will address, protect, and restore biodiversity, including for common native species, by management action. One of the goals of the BFFIP is to reduce invasive and weed species through various methods. Reducing invasive weeds to allow native species to diversify will benefit biodiversity. Invasive weeds disrupt the ecology of natural ecosystems by displacing native plants and the animal species that depend upon them, reducing native biodiversity. Next to habitat loss, over 50 percent of the loss of native biodiversity globally has been attributed to introduced species, and nearly half of the species listed as threatened or endangered under the Endangered Species Act in the U.S. are at risk due to competition with alien or introduced species (Zevit, 2019). Invasive species reduce biodiversity by taking over ecosystems with one plant and habitat type, often replacing numerous other native species and reducing food sources that support a diversity of wildlife. It should also be noted that, as stated in **Master Response 4: Wildlife,** CEQA does not require the analysis of impacts to common species, except as it pertains to migratory species and corridors. One of the goals of the BFFIP is to reduce invasive and weed species through various methods. Reducing invasive weeds to allow native species to diversify will benefit biodiversity. Common species, including small mammals and reptiles, will benefit from increased biodiversity.

The impacts and corresponding mitigation in Section 3.3 Biological Resources of the Draft EIR are presented by management action. Common native species, except migratory species, do not need to be addressed under CEQA, but sensitive communities, plants, and wildlife are addressed and the measures to minimize effects to these resources is presented in Section 3.3.7 of the Draft EIR.

Response to Comment A7-7

The commenter states the plan only protects “significant biological resources” and does not even mention wildlife. Refer to Response to Comment A7-6 for a discussion of where and how the March 2019 Draft BFFIP addresses biodiversity and wildlife. The commenter states that the plan should address all taxa that depend on the project area. Identifying all taxa that could occur in the plan area is not necessary for the definition of the plan since the plan is focused on protecting structural and natural resources from the effects of wildfire and reducing invasive species. The prescriptions in the plan would not change given a broader list of all common species that could occur in the area. The focus is on key habitats and maintaining those habitats. While the plan does not identify in detail all species and habitats in the area, including acreages and locations, the Draft EIR does include this information. The Draft EIR identifies all habitats within the plan area, including all plant communities, their acreages, and the common plant species found in that community (see Table 3.3-1 on pages 3.3-4 through 3.3-8 of the Draft EIR). Page 3.3-22 of the Draft EIR adequately explains the consideration of wildlife species in the Draft EIR as follows:

> The extent and diversity of habitat on District lands supports numerous mammal, bird, reptile, amphibian, and invertebrate species. A matrix of relatively undisturbed habitats is present, including coniferous forests, oak woodland and savannah, mixed hardwood forests, riparian woodland, chaparral, coastal scrub, grasslands, and aquatic and wetland habitats. These lands are used by numerous common wildlife species as movement, foraging, and breeding habitat. A comprehensive assessment of all wildlife species potentially occurring on a particular site can be difficult to both obtain and confirm because some species only occur in a particular area for a short period (such as during migration or dispersal from natal birthing areas), some are inactive during one or more seasons, and some are nocturnal or reclusive in nature. Therefore, the following discussion is intended to provide a general characterization of the types of common wildlife species occupying District lands and is not a comprehensive list of all wildlife species present. Appendix F provides more comprehensive lists of mammal, bird, reptile, and amphibian species known or likely to occur on District lands.

Response to Comment A7-8

The comment states that there is no mention of wildlife in the BFFIP, except for northern spotted owl, and that the Draft EIR needs to discuss potential impacts of management actions on all wildlife. Refer to Response to Comment A7-7 for a discussion as to why the plan does not need to identify all wildlife species that could occur in the plan area and the focus of the plan on protecting habitats from wildfire and enhancing biodiversity. Refer to **Master Response 4: Wildlife** for a discussion of the scope of the biological resources' analysis in accordance to CEQA, including common wildlife species. CEQA does not require the analysis of impacts to common species except as it pertains to migratory species and corridors. Impacts to special-status wildlife species are less than significant with mitigation.

Response to Comment A7-9

The commenter asks how the plan will avoid further declines in wildlife populations caused by fuel reduction and the failure to remove invasive species. CEQA does not require the analysis of impacts to common species except as it pertains to migratory species and corridors. Impacts of the plan on migratory birds are addressed in the Draft EIR as required under CEQA. The Draft EIR includes a discussion of impacts to nesting birds from plan implementation and includes mitigation (MM Biology-7 on page 3.3-128 of the Draft EIR) to reduce impacts. As stated in Response to Comment A7-3 and in **Master Response 1: Definition and Location of New and Expanded Fuelbreaks**, a fuelbreak is not a denuded area without trees or other vegetative cover. While the work alters the habitats, it does not destroy or completely remove the habitat.

Fuel reductions would not result in declines in wildlife populations as treating invasive species and forest diseases would allow for expansion of native plants and increased diversity. The plan would therefore not result in an increase in invasive species over baseline conditions that could impact wildlife.

Response to Comment A7-10

The commenter requests more information on the Point Blue 2014 monitoring of common species. Point Blue Conservation Science (Blue Point) monitored the abundance of land-birds on District lands from 1996 through the present and analyzed trends in abundance for 44 species. More information on this monitoring and results can be found on page 3.3-22 of the Draft EIR. This reference was cited for the data that it provides on general trends of the populations of common species on the Watershed, which can give some insight into common avian species' response to on-going vegetation management. Common avian species in the study utilized mixed evergreen hardwood forest, oak woodland/savannah, coast redwood forest, chaparral, and grassland/edge habitats. Most if not all habitats on the Watershed thus would have been exposed to existing management activities over the timeframe of the study. Species were chosen that are common on the Watershed and whose populations are easy to monitor. The study did not include waterbirds, shorebirds, owls, non-breeding species, and species not well sampled within the point-count method such as non-territorial species, flocking species, and species with very large territories.

Additional information on common species is not necessary to identify the actions of the plan, nor for the environmental analysis, since CEQA does not require the analysis of impacts to common species except as it pertains to migratory species and corridors. It is noted that additional information is available in the 2014 Point Blue study that has not been presented in the Draft EIR; however, inclusion of this information would not result in any changes to the analysis presented in the Draft EIR nor change the conclusions reached.

The commenter also requests the measures to be taken to reverse declines of species such as pileated woodpecker and Stellar jay. It is not the function or intent of the BFFIP to reverse the existing declines of species such as pileated woodpecker and stellar jay since the program is a vegetation management plan.

Response to Comment A7-11

The commenter states that destruction of habitat for many native wildlife species should be identified as a significant impact and mitigation identified to reduce effects from leaving broom and from removing understory habitats for fuelbreaks and to reduce fuel loads.

Destruction of habitat would not result from implementation of the BFFIP. The plan would result in a decrease in invasive species as compared with existing or baseline conditions and a decrease in catastrophic fire risks, contrary to the statement made by the commenter. Many of the native species listed by the commenter are common species. CEQA does not require the analysis of impacts to common species except as it pertains to migratory species and corridors. Impacts to special-status wildlife species (such as the avian species listed by the commenter that are protected under the Migratory Bird Treaty Act) are less than significant with mitigation. As stated in Response to Comment A7-3 and in the **Master Response 1: Definition and Location of New and Expanded Fuelbreaks**, a fuelbreak is not a denuded area without trees or other vegetative cover. While the work alters the habitats, it does not destroy or completely remove the habitat such that habitat loss would occur. Since impacts would not be significant with regard to loss or destruction of habitat, CEQA does not require the application of mitigation. See Section 3.3 Biological Resources, of the Draft EIR, for the 17 mitigation measures defined to minimize effects on habitat and species to less than significant levels.

Response to Comment A7-12

The commenter requests an impact analysis that addresses all ground-dwelling species and species that depend for some part of their life cycles on the habitats that will be impacted by the BFFIP. Refer to **Master Response 4: Wildlife** for a discussion of the scope of the biological resources' analysis in accordance with CEQA, including common avian wildlife species and ground-dwelling species. As stated in Response to Comment A7-3 and in the **Master Response 1: Definition and Location of New and Expanded Fuelbreaks**, a fuelbreak is not a denuded area without trees or other vegetative cover. While the work alters the habitats, it does not destroy or completely remove the habitat. Habitat for ground-dwelling species would remain. Existing and new fuelbreaks would only constitute approximately four percent of the plan area. Fuel reduction zones would reduce invasive species and diseased species but would not alter habitat such that it could not be used by nesting (either in trees or on the ground) birds. The commenter asks how the District will ensure that there are no habitat reductions for declining species. Refer to Response to Comment A7-10, which explains why the BFFIP does not address currently declining species and how the EIR addresses migratory birds. Impacts to migratory bird species are mitigated to less than significant.

Response to Comment A7-13

The commenter states that there are no population trend analyses for mammals, reptiles, or amphibians that could be impacted by the loss of understory habitat. The comment references the Draft EIR's discussion of common wildlife species. Population trend analyses for common species has not been included. Refer to **Master Response 4: Wildlife.** The CEQA Guidelines do not contain a question regarding substantial adverse effects on all common species. Impacts to common species is not required under CEQA beyond impacts to migratory species and corridors. While impacts to common species are not directly addressed, the BFFIP would reduce invasive species and improve forest health. Fuelbreaks result in some alteration of habitat, but habitat would remain as fuelbreaks do not result in the denuding of the habitat. Refer to **Master Response 1: Definition and Location of New and Expanded Fuelbreaks.** New fuelbreaks would constitute approximately 0.5 percent of the plan area, and new and existing fuelbreaks constitute four percent of the plan area. While the loss of some ground cover in fuelbreak areas (and to a lesser extent in fuel reduction areas) would change ground cover for common species, surrounding areas would remain with abundant cover.

Response to Comment A7-14

The commenter's opinion regarding the mitigation measures' ability to reduce significant effects is noted; however, the comment lacks detail as to what is lacking in the measures to enable a more detailed response.

The commenter states that 20 special-status bird species are listed as nesting on District lands, but there is no discussion about the impacts to these species, except northern spotted owl. As stated in Responses to Comments A7-3 and A7-12, and in **Master Response 1: Definition and Location of New and Expanded Fuelbreaks**, a fuelbreak is not a denuded area without trees or other vegetative cover. While the work alters the habitats, it does not destroy or completely remove the habitat for migratory birds (see **Master Response 4: Wildlife**). The Draft EIR addresses special-status bird species and impacts to their habitat. Nesting birds would continue to have abundant areas to nest (see page 3.3-77 of the Draft EIR). New fuelbreaks would constitute approximately 0.5 percent of the plan area and would not have substantial impacts on nesting habitat for birds.

Response to Comment A7-15

The commenter asks how much of the northern spotted owl activity centers would be subject to understory clearing for fuel reduction or fuelbreaks and now much is native vegetation. Refer to **Master Response 4: Wildlife** for a summary of the analysis of impacts on northern spotted owl. Table 2.7-1 in Chapter 2 Project Description provides the maximum annual acreage of fuelbreak maintenance and fuel-load reduction on District lands. At full plan build-out, approximately 2,000 acres per year of vegetation management actions could occur, which represents about nine percent of the total plan area, most of which is in the Watershed (versus Nicasio or Soulajule Reservoirs). Treatments proposed within a northern spotted owl activity center in any given year would only affect vegetation within a portion of the total 5,956 acres of activity centers present on District lands (5,581 acres within the Watershed). For example, of the 117 acres of new or widened fuelbreaks proposed for construction over the life of the BFFIP, approximately 58 acres are within 0.25 mile of a known northern spotted owl activity center. New fuelbreak creation is the primary activity that would impact native vegetation, so up to 58 acres could be within 0.25 mile of an activity center. As stated in the master response, complete removal of native plant and habitat communities would not occur, so while the activity could result in alteration of habitat, it is only a small fraction of habitat and activity center that could be impacted (approximately one percent for fuelbreaks, for example). Fuel reduction zones could impact up to another 2,200 acres over the life of the plan, but in these areas, habitat and thinning would be much less intensive than fuelbreaks and would focus on invasive species removal and forest disease treatment, which would benefit habitat.

The comment is correct that the Draft EIR acknowledges that fuelbreak creation could impact northern spotted owl and dusky-footed woodrat through removal and thinning of vegetation, and noise could also impact northern spotted owl. Refer to **Master Response 4: Wildlife,** the section entitled "Impacts to Northern Spotted Owl from Habitat Loss and Loss of Prey Base" for a discussion of the longer-term impacts to northern spotted owl from habitat alteration for the creation of fuelbreaks and why the impacts are less than significant. The master response also discusses impacts to dusky-footed woodrat and explains how impacts to the northern spotted owl prey are reduced to less than significant.

The commenter questions the statement that vegetation removal would promote the growth of a diversity of vegetation, which supports a wider prey base. The commenter's disagreement with the statement that the BFFIP will promote growth of a diversity of vegetation is noted, but not valid. Removal of invasive species and forest disease over baseline conditions would improve habitat and species diversity because it would allow native species to grow and support the diversity of wildlife that invasive species cannot. The commenter notes that northern spotted owl primarily feed on woodrats and that vegetation removal in fuelbreaks would result in more broom and reduced diversity. Several management actions include the treatment of broom and weed species, and therefore weed populations would not increase as a result of the plan's implementation.

The commenter states that mitigation addresses construction but that longer-term impacts of removing vegetative habitats must also be addressed. The analysis and mitigation also address long-term habitat impacts on northern spotted owl (and other special-status species). Refer to page 3.3-27 of the Draft EIR, under the heading ***Indirect (Habitat) Impacts from Various Vegetation Management Techniques***. MM Biology-14 requires that

> areas proposed for vegetation management within 0.25 mile of a northern spotted owl "activity center maintain a mix of disturbed (i.e., under active vegetation management) and undisturbed habitat (i.e., not under active vegetation management), and avoidance of woodrat stick nests, to minimize impacts on northern spotted owl from diminished prey populations. If existing woodrat nests are avoided, impacts on prey (woodrat) density should not be affected; a study of dusky-footed woodrats in the redwood region of California did not find an association between abundances of woodrats and different intensities of forest thinning (Hamm & Diller, 2009). With implementation of mitigation, short-term, indirect impacts from habitat alteration on northern spotted owl would be less than significant.

MM Biology-14 has been revised to state, under item 2, that woodrat stick nests and the areas around the nests would be avoided during vegetation management activities, as shown in Chapter 3.

The commenter states that the "prolific northern spotted owl population in the State" on Watershed lands has no connection to impacts from fuelbreaks. The Draft EIR does not state that fuelbreak maintenance or creation has no impact, but rather that the population on District lands is doing better than other populations in California although fuelbreak maintenance occurs annually and has since 1995 (see page 3.3-8 of the Draft EIR4). This observation supports a conclusion that any ongoing environmental effects from fuelbreak maintenance are not at such a level that the population is doing more poorly than other populations where fuelbreaks may not maintained. Therefore, the Draft EIR concludes that on-going maintenance of fuelbreaks, defensible spaces, and roadside areas would not result in a significant permanent change to the existing habitat conditions (see page 3.3-84 of the Draft EIR, p. 3.3-84).

Response to Comment A7-16

The commenter asks to explain why activities may need to occur during the nesting season, as stated in MM Biology-8. Allowing work to occur during the nesting season allows for more treatments to occur to meet BFFIP goals and to reduce fire hazards and invasive species that impact habitat and ecosystem health. The mitigation measure identifies the methods to implement if work occurs during the nesting season of northern spotted owl to avoid impacts to the species (see page 3.3-129 of the Draft EIR). The commenter states that the nesting season dates should be modified to extend from February 1 through July 31; however, these are the dates that are identified in the mitigation measure already. Trees with nests would be avoided during this period per item 1 in MM Biology-8. If the young have fledged, work can occur closer to the nest trees as young would not be impacted by the work.

The commenter questions the long-term impacts of performing work even outside the nesting season, near areas that support northern spotted owl nesting. Refer to **Master Response 4: Wildlife** and Response to Comment A7-15 for a summary of the analysis of impacts on northern spotted owl, including the longer-term impacts from limited habitat alteration in fuelbreaks. As stated in the master response, complete removal of native plant and habitat communities would not occur and impacts to habitat would be minimal and less than significant given the limited amount of work (new fuelbreaks comprise 0.5 percent of the plan area), and the fact that habitat is not destroyed or removed, only altered.

The commenter states that the second option in MM Biology-8 to use USFWS guidance should be dropped because it is based on the lumbering areas of the Pacific northwest. The lumber areas in Northwestern California include large tracts of Douglas-fir trees, which are a primary timber tree species. The northern spotted owl habitat found in Northwestern California is similar to the suitable habitat found on District lands, as evidenced by Douglas-fir forests and presence of the owl. Due to these similarities, use of the guidance and methodology in the USFWS "Transmittal of Guidance: Estimating the Effects of Auditory and Visual Disturbance to Northern Spotted Owls and Marbled Murrelets in Northwestern California" as required by MM Biology-8 is appropriate.

Response to Comment A7-17

The commenter states that MM Biolgoy-14 is not an avoidance measure. MM Biology-14 is not meant to be avoidance of northern spotted owl core habitat as the commenter suggests. The measure is to ensure avoidance of nesting birds. The commenter also asks why MM Biology-14 does not require consultation with GIS data collected by Blue Point on an annual basis. Point Blue data is, in fact, annually incorporated in the District's GIS data that would be consulted as a requirement of MM Biology-24 and covers both MMWD land and Marin County Parks lands (formerly Marin County Open Space District). Any additional studies beyond those conducted by Point Blue would also be in the District's GIS. The commenter states that 2017 and 2018 Blue Point data on the locations of northern spotted owl activity should be included in the Draft EIR.

The Draft EIR utilized the 2016 Blue Point data for northern spotted owl and is presented in Figure 3.3-19 on page 3.3-55. The data includes activity centers on MMWD and surrounding lands. The figure shows the populations on MMWD land as well as surrounding lands. The exact locations of the northern spotted owl activity centers are not presented in order to protect the species (the map is a small-scale view). The purpose of the figure is to give the reader a sense of the distribution and intensity of the spotted owl activity centers. While newer data has become available for 2017 and 2018, it would not change the analysis and conclusions of the Draft EIR nor the mitigation that requires annual updating of the northern spotted owl activity and avoidance of areas. Northern spotted owl activity will change to some degree annually.

Response to Comment A7-18

The commenter states that the EIR should analyze the areas that would be avoided (presumably because they are in northern spotted owl activity centers) and compare those areas with the planned fuel reduction and fuelbreak areas. The commenter also notes that areas where northern spotted owl nest should not be treated at all to avoid long-term impacts to habitat. Refer to Response to Comment A7-15 for a discussion of the acres of northern spotted owl habitat that could be impacted by the plan and why impacts to that habitat are less than significant, including the fact that habitat is only altered and not destroyed or removed and that the amount of habitat affected is minimal compared with the overall available habitat. There will be no long-term loss of northern spotted owl habitat. MM Biology-8 also requires that steps be taken annually to identify, during that year, the locations of northern spotted owl nesting activity and to avoid fuel treatment in nesting areas until the young have fledged or September 1st, which reduces impacts to less than significant.

Response to Comment A7-19

The commenter asks what is meant by "a mix of disturbed and undisturbed habitats" and points out that maintaining a healthy prey base (i.e., woodrats) is critical for owl survival. Refer to Page 3.3-78 of the Draft EIR, which identifies what is meant by "disturbed and undisturbed habitats," which is not vague:

> MM Biology-14 requires areas proposed for vegetation management within 0.25 mile of a northern spotted owl activity center maintain a mix of disturbed (i.e., under active vegetation management) and undisturbed habitat (i.e., not under active vegetation management).

The commenter asks if the District knows where woodrat nests are located. Woodrat nest locations are not currently well known or mapped because this data has not been collected in the past. See **Master Response 4: Wildlife** under the section "Impacts to Northern Spotted Owl from Habitat Loss and Loss of Prey Base" for a discussion as to the impacts to prey base and how they are reduced to less than significant with mitigation. As stated in M**aster Response 4: Wildlife**, woodrats prefer to build nests in dense chaparral and in areas near streams. These areas generally do not correspond to new fuelbreak areas. Population declines are not anticipated as few nests are expected to be impacted given the location of fuelbreaks and the limited acreage that would be impacted as compared with the plan area (0.5 percent of plan area is new or expanded fuelbreak).

The commenter also asks what habitat mix is adequate and why northern spotted owl activity centers must be disturbed at all. MM Biology-14's purpose is primarily to ensure that an entire activity center is not being treated at one time. Vegetation treatment in activity centers would be designed to improve the habitat, and the habitat mix is described in MM Biology-17, which states:

> Opportunities to conduct vegetation management to enhance development of late-successional characteristics or to meet other restoration goals in a manner compatible with retaining resident northern spotted owls shall be evaluated and implemented. Restoration activities conducted near northern spotted owl sites shall first focus on areas of younger forest less likely to be used by northern spotted owls and less likely to develop late-successional forest characteristics without vegetation management. Vegetation management projects shall be designed to include a mix of disturbed and undisturbed areas, retention of woody debris, and development of understory structural diversity to maintain small mammal populations across the landscape.

The acres of habitat to be treated would be determined annually. As previously stated, there will be no long-term loss of northern spotted owl habitat.

Response to Comment A7-20

The commenter states a concern over clearing of vegetation around woodrat nest structures. The commenter states that avoidance of the woodrat nests is not enough to ensure survival of the woodrats as clearing would leave the woodrats vulnerable to predation and/or reduced food. Avoidance of nests would reduce impacts, but some impacts could remain for nests in fuelbreak areas that may clear ground vegetation within their foraging range, as the commenter suggests. However, the overall population of woodrats is not anticipated to be significantly impacted. Impacts to woodrat populations and declines in woodrat populations are not anticipated due to the limited amount of habitat alteration resulting from plan implementation as compared with the Watershed size, as described in **Master Response 4: Wildlife**. As stated in Responses to Comments A7-13 and A7-14 and the master response, approximately 0.5 percent of the Watershed would be subject to new or expanded fuelbreak construction. It should also be noted that woodrats prefer to build nests in dense chaparral and in areas near streams. These areas generally do not correspond to new fuelbreak areas (see Figure 2.3-1 in the Draft EIR). Woodrat population declines are therefore not anticipated as few nests are expected to be impacted by clearing, given the location of fuelbreaks and the limited acreage that would be impacted in comparison to the overall plan area.

The commenter asks how woodrat nests would be identified and whether pre-project surveys of woodrats would be required. Under MM Biology-14, the most recent data with northern spotted owl activity centers would be consulted and woodrat nests would be avoided through surveys by a qualified biologist. MM Biology-14 has been revised to state under item 2 that woodrat stick nests and the areas around the nests would be temporarily demarcated during surveys and avoided during vegetation management activities, as shown in Chapter 3**.**

The comment is noted regarding northern spotted owl foraging, hunting, and dispersal habitat. Habitat alterations would be limited as compared with overall foraging habitat available for northern spotted owl in the plan area.

Response to Comment A7-21

The commenter states that mitigations proposed for special-status species are the same for most species and includes conducting surveys and avoiding work during the nesting or breeding season if the species is nesting. This assessment of the mitigation is generally accurate.

The commenter raises additional questions about badgers and why mitigation allows for relocation of badgers, and whether this method has been used before and with what success. As stated in MM Biology-6 in the Draft EIR, badgers would only be passively relocated, which involves use of one-way doors to burrows, allowing the badgers to exit the den but not reenter. The badger would choose a new location for a den. Badgers would not be actively relocated. Passive badger relocation is an acceptable measure, but only under the direction of a qualified biologist and with approval of CDFW, who can assess whether the relocation is appropriate and can be done in such a way that the badger is not impacted. These methods have likely been used before with success since they are allowed by CDFW. The provision to allow work within the 100-foot buffer has not been removed because the measure only allows the buffer to be reduced when a biologist determines that its reduction would not have a significant impact on the badger. The measure allows buffers to be modified by the qualified biologist, provided the badgers are protected, and the buffer should not be removed until the qualified biologist has determined that the den is no longer in use. As stated, the measure is protective and reduces impacts to less than significant.

Response to Comment A7-22

The commenter asks for further discussion of impacts to pond turtles, which can burrow into the mud during overwintering and, therefore, could be crushed. It is feasible that western pond turtle hatchlings overwintering in burrows near ponds could be crushed from use of heavy equipment. Additional measures have been added to MM Biology-9 to protect overwintering pond turtle hatchlings in nests. Refer to Chapter 3 for revisions to MM Biology-9.

Response to Comment A7-23

The commenter questions why mitigation requirements for California red-legged frog and foothill yellow-legged frog are different. Both mitigation measures for California red-legged frogs and for foothill yellow-legged frogs require surveys. MM Biology-12 allows for foothill-yellow legged frogs to be relocated, but MM Biology-10 does not allow red-legged frog to be relocated. The reason for the difference is that each species has different sensitivities and listing statuses, with a higher listing status of *endangered* for red-legged frog. Relocation is allowed for either species under the appropriate permits; however, as a conservative precaution, MM Biology-12 has been modified to also require that no work commence until the yellow-legged frog has moved on its own, same as for red-legged frog, since yellow-legged frog is a Candidate species and will now also require avoidance. The revisions are shown in Chapter 3 of this Final EIR.

Response to Comment A7-24

The commenter asks why relocation of California giant salamander is allowed and states that it should not be allowed. Relocation of California giant salamander is an appropriate mitigation measure and a standard practice conducted by qualified biologists, as described in Response to Comment A2-17. Should a California giant salamander be encountered during work, MM Biology-17 allows for a qualified biologist to safely and legally guide the individual California giant salamander out of harm's way or to avoid the area. The mitigation serves to preserve and prevent injury to any individuals encountered. Impacts to California giant salamanders would be less than significant with the mitigation.

Response to Comment A7-25

The commenter asks for the conditions under which removing trees with active bat maternity nests would be needed. As noted in MM Biology-5, certain roost or maternal trees used by bats may be identified as hazardous or unsafe, necessitating removal. A tree could be considered hazardous if it is diseased or dead or could pose a hazard to workers or recreationalists. The recommendation to not remove maternal nests is noted, but no change has been made in MM Biology-5 as tree removal may be needed to address hazards and ensure the safety of workers.

Response to Comment A7-26

The commenter asks what work could adversely impact wetlands, including seasonal wetlands, and states that work that could adversely impact wetlands should be avoided. The commenter notes that filling, draining, or modifying wetlands and vegetation require permits. The comment is noted. Impact Hydrology-1 analyzes impacts on streams and wetlands. Alterations to either intermittent or perennial streams or to wetlands would generally be avoided, but if avoidance is not possible, work may require a Fish and Game Code Section 1602 Lake and Streambed Alteration Agreement and potentially a permit under Section 404 of the Clean Water Act and Section 401 Water Quality Certification from the San Francisco Regional Water Quality Control Board prior to performing the work. These permits are also listed in Table 2.15-1 in Chapter 2 Project Description (page 2-59 of the Draft EIR).

Response to Comment A7-27

The commenter states that the District should commit now to preparing an EIR or Mitigated Negative Declaration for restoration projects. Refer to Response to Comment A3-8 for information about the level of information available for MA-26 and MA-27 and the future evaluations that would be conducted to determine whether further environmental review is required. It is not feasible at this time to determine the level of environmental review that would be required for these two management actions. The type of CEQA document required would depend on the details of the plans prepared for the restoration projects.

Response to Comment A7-28

The commenter asks how many of the 19 "administrative" actions are new activities and how many are continuations of on-going activities. Refer to Chapter 5 of the March 2019 Draft BFFIP for a description of the 19 administrative management actions. MA-1 through MA-7 are inventory actions that are currently underway, and the BFFIP prescribes completion of those inventories. MA-8 through MA-15 and MA-18 include management actions to continue working with neighboring jurisdictions and to revise and update toolboxes for vegetation management and best management practices and to conduct project planning. MA-16, MA-17, and MA-19 include new monitoring actions. These actions would result in no new environmental effects.

The comment is generally correct that proposed management actions with potential for environmental effects generally encompass maintaining fuelbreaks, creation of new or expanded firebreaks, removing SOD, reducing fuels, prescribed burning, and removal of invasive species.

Response to Comment A7-29

The commenter requests all new and expanded fuelbreaks to be shown on a figure. Refer to **Master Response 1: Definition and Location of New and Expanded Fuelbreaks** for a discussion of the figures that show the new and expanded fuelbreaks**.** New and expanded fuelbreak locations are shown in Figure 2.7-1 of the Draft EIR. Figures 2.6-1 through 2.6-4 have been revised to show the new and expanded fuelbreaks and their zoning in addition to the existing fuelbreaks and their zoning. The fuelbreaks are not shown to scale due to their narrow widths as compared with the overall Watershed size, but the relative position of the new and expanded fuelbreaks as compared with the existing fuelbreaks is shown.

The commenter requests that the discussion of each new and expanded fuelbreak describe whether the expanded area is vegetated with native or invasive species and what species of wildlife depend on the habitats where vegetation will be removed. The commenter also requests a discussion of habitat impacts from removal of vegetation and the acreage of new fuelbreak areas. The new and expanded fuelbreaks are almost entirely expansions of existing fuelbreaks. The condition of the existing fuelbreaks are shown in Figures 2.6-1 through 2.6-4 in Chapter 2 of the Draft EIR, based on the classification of the fuelbreaks as either "optimized," "transitional," or "compromised." The description of each classification and the level of invasive and native species identified in the each fuelbreak category is presented in Section 3.5.2 of the BFFIP. Figures 2.6-1 through 2.6-4 of the Draft EIR have been revised to show new fuelbreak areas (see Chapter 3 of this Final EIR). The extent of invasive species in these areas can be discerned by the classification of the adjacent fuelbreak. Invasive species would be removed when new or expanded fuelbreaks are created. Refer to **Master Response 1: Definition and Location of New and Expanded Fuelbreaks** for a discussion of the impacts and to species from new fuelbreaks and the total acreage of new fuelbreaks and mapping. Habitat would not be destroyed or lost. The acreage of new fuelbreaks is presented in several places in the March 2019 Draft BFFIP and the Draft EIR, such as on page 2-36 of the Draft EIR. New fuelbreak areas are generally expansions of existing fuelbreaks and are shown on revised figures 2.6-1 through 2.6-4 in Chapter 3 of this Final EIR. One-hundred seventeen acres of new fuelbreaks would be constructed. Impacts to habitat and wildlife from fuelbreak construction is presented on pages 3.3-97 to 3.3-98 and 3.3-114 to 3.3-115 of the Draft EIR. The commenter asks again for the criteria used to decide which fuelbreaks to expand. Section 3.4 on pages 3-17 through 3-18 of the March 2019 Draft BFFIP discusses how the District assessed fuelbreak needs and locations, based in part on an assessment of risks, GIS analysis, and the location of the WUI. Choice of fuelbreak locations is not arbitrary as the commenter states. This discussion addresses the criteria used to decide which fuelbreaks to expand and the location of fuelbreaks, which includes the location of the WUI and includes providing access to fight fires (so generally along existing access roads). Fuelbreaks are intended to slow fires and provide a place from which fires can be fought. It is difficult to know the intensity of fire that the fuel breaks will assist in reducing as fire spread depends on many factors including wind direction, flame lengths, and climatic and weather conditions. The fuelbreaks are designed to allow for an area within which firefighters can access the fire and that can slow spread of the fire before it reaches the WUI.

Response to Comment A7-30

The commenter states that maintenance of fuelbreaks will only occur every five years. This assertion is incorrect. Refer to Section 3.5.2 of the March 2019 Draft BFFIP on pages 3-26 through 3-40 for a description of the treatments by type of fuelbreak, including frequency. Optimized fuelbreaks would be treated once every three to seven years. Transitional fuelbreaks would also be treated every three to seven years with annual, focused weed control. Compromised fuelbreaks are treated with annual brushing to cut back invasive species. On-going weed control is part of the plan.

Response to Comment A7-31

The commenter states that the fuel reduction zones are not discussed, nor criteria identified, nor figures shown identifying their locations. The comment is incorrect. Refer to **Master Response 1: Definition and Location of New and Expanded Fuelbreaks** for the definition of a fuelbreak and description of WAFRZ. The master response provides information pertaining to the existing and proposed fuelbreaks and WAFRZ. The WAFRZ could be built within the Ecosystem Restoration zone, as shown in Figures 3-16 through 3-19 of Chapter 3 of the BFFIP and Figure 2.6-6 to Figure 2.6-10 on pages 2-26 to 2-30 of the Draft EIR. The Ecosystem Restoration/WAFRZ are described on page 2-31 of the Draft EIR.

The criteria for designating WAFRZ and the type of work that would occur in them, and the goals of the work is described on page 2-31 of the Draft EIR, is as follows:

> The restoration/wide area fuel reduction zones (WAFRZ) share many of the characteristics of restoration zones in terms of natural habitat but are distinguished by their proximity to existing infrastructure and the presence of natural resources considered at high risk of permanent degradation int eh event of a high intensity wildfire. The District’s goals in this zone include both ecosystem improvement and wildfire risk reduction for both natural resources and human infrastructure. The District’s biological and wildfire goals are not met within these areas at this time, but significant gains are possible. Therefore, the long-term strategy is to increase effort to achieve measurable improvements in both fuels profile and ecosystem health through invasive species removal and forest management.

Fuel reduction in WAFRZ is described further in the BFFIP, page 2-9, and states:

> The other half of fuel load reduction acreage includes work conducted within wider areas of habitat and adjacent to infrastructure-bordering fuelbreaks. The District has reduced accumulated fuels across grassland, woodland, and forest habitat in these wider areas to achieve a combination of wildfire risk reduction and habitat enhancement (e.g., invasive weed control).

The commenter asks how the fuel reduction will contribute to forest health. The commenter also asks whether fuel reduction zones would be in strategic locations that reduce fire hazards and disease and asks how whether removal of diseased trees would reduce disease. See response to Comment A7-20 for more information. The work within the WAFRZ would contribute to forest health by reducing invasive species, in particular, but also SOD and other accumulated fuels. Removal of SOD-infected trees would reduce the presence of the disease. WAFRZ are generally along fuelbreaks and expand the area from which fires can be slowed and invasive species treated, and so would be strategic for the reduction of fire hazards. Fuels reduced in WAFRZ would include some native, invasive, understory shrubs, forbes, downed trees, sticks, and leaves, as the commenter suggests, and similar to a fuelbreak, but with less intensity. Refer to **Master Response 4: Wildlife** for a summary of impacts on wildlife, e.g. the dusky footed woodrat, from fuelbreak creation and maintenance as well as other proposed activities, including fuel reduction zones.

WAFRZ could increase the amount of areas with reduced vegetation. Page 3-26 of the BFFIP identifies that the District's system includes 2,650 acres of WAFRZ, of which 450 have been treated between 1995 and 2015. Up to 2,200 more acres could be treated. The commenter asks how woodrat houses would be protected in these areas. The impacts to woodrat and other wildlife species would be less than significant since treatment would focus on removal of invasive species and forest disease. Some fuel density reduction would occur but would be less intense than for fuelbreaks. Habitat would remain for avian species, special-status species, and woodrats (as prey for northern spotted owl).

Response to Comment A7-32

The commenter asks for the current status of SOD on the Watershed and whether it is increasing or decreasing. Figure 2.4-1 and Figure 2.4-2 show the spread of SOD from 2004 to 2014 within the Mount Tamalpais Watershed. SOD has been increasing across District lands. The acres of impact on Figure 2.4-2 have been revised as shown in Chapter 3. Environmental effects on plant communities and wildlife species as a result of SOD is a part of baseline conditions (CEQA Guidelines Section 15125). Research on the effect of removing SOD-infected trees does exist. One recent paper by scientists from Oregon State University looks at different methods of treatment of SOD infestations and their effectiveness. The paper concludes "that eradication of SOD from infested sites is difficult but not impossible. The disease usually does not persist after cutting infected trees" (Hansen, 2019). It follows the logic that removal of the tree removes the pest and reduces its ability to spread.

The commenter asks if it is the District's intention to remove all or a portion of the trees infected with SOD and, if a portion, what portion. MA-23 addresses how the District would address SOD-impacted forests. Dead and dying trees would be removed to reduce fuel loads. The District cannot remove all trees infected with SOD as it does not have the resources to do so given the extent of SOD (covering over 11,000 acres of the Watershed in 2014, as shown in Figure 3-9 of Chapter 3 of the BFFIP). According to Chapter 7 of the BFFIP, Table 7-2, up to 60 acres of fuel reduction (an unspecified subset of which could be SOD treatment) would be treated per year at full implementation.

The commenter asks for a description of the habitat and wildlife impacts of the spread of SOD. The BFFIP would not result in the spread of SOD; therefore, the impacts of the spread of SOD are outside the scope of CEQA and the EIR as they are baseline.

The commenter asks for a discussion of the ecological benefits of leaving some dead and dying trees in a forest and how the massive removal of dead trees would impact biodiversity, forest ecosystems, and resilience. As previously stated, the District would not be able to remove all dead trees impacted by SOD. Over 11,000 acres have been impacted. Some amount less than 60 acres per year would be treated, and likely just a few acre plots per year. Massive removal of dead trees would not occur. The commenter requests that the benefits of dead and dying trees to insects and to foraging and nesting birds and animals be described. Some dead and dying trees provide habitat; however, the plan would not remove all dead and dying trees, as previously stated. Since many dead and dying trees would remain, the benefits would remain. Overall, treatment of SOD and removal of dead and dying trees that spread the disease would have benefits by creating more healthy habitats, with living trees that provide food, shelter, and moisture to special-status and other wildlife species.

The commenter asks what "disease resistant" species would be used in restoration. Page 2-38 of the Draft EIR states that "native conifer and hardwood species" would be used to replace diseased tan oak. The appropriate species to plant would be selected dependent upon existing habitat and site conditions (some that do grow in the area of tanoak disease and removal).

The commenter asks how different replacement trees would change the natural conditions of the habitat. Habitats would generally be maintained to those before disease (i.e., would remain native conifer and/or hardwood forest). Wildlife populations, therefore, would not be impacted and would utilize the areas in the same ways. The treated areas would have improved ecosystem functions.

The commenter asks how decisions are made about trees to be removed or retained. These decisions would be made by District-qualified staff in the annual plan prior to treatment. The decisions would be based on resources and areas where success can be attained. The District has been conducting studies and monitoring treatment sites as part of the Resilient Forests Project and other projects. The knowledge gained from historic and ongoing SOD treatments was incorporated into and would be implemented as part of the BFFIP. Annual performance criteria for MA-23 are identified in Table 2.7-1 in Chapter 2 Project Description in the Draft EIR.

The commenter states that the only discussion of treatment of SOD is removing infected trees and asks if there are treatments that have been known to cure or halt SOD infections and if the District has considered chemical treatments. The commenter is correct that the primary method of SOD treatment addressed is removal and restoration of the sites with more resilient trees. The plan does not include chemical methods of control but does not rule out their use. Separate environmental review would be required. If chemical controls are effective and depending on the environmental impacts of the chemical methods, they could be incorporated into the plan via amendments and after additional CEQA review.

The commenter's description of 20 annual acres of treatment under MA-23 does not align with any of the data presented. MA-23 includes 60 acres of work, as identified in Table 7-2 on page 7-5 of the BFFIP. The commenter asks how the areas are chosen for action. As previously stated, decisions regarding which acreages to treat within the designated zone, for up to 60 acres per year, would be made by District-qualified staff in the annual plan prior to treatment. The decisions would be based on resources and areas where success can be attained.

The District has been conducting studies and monitoring treatment sites as part of the Resilient Forests Project and other projects, as the commenter notes and as noted above. The District's ongoing SOD project for forest resilience had some results available in 2017 (Cobb, et al., 2017). The pilot study included a stand-level restoration experiment on Mount Tamalpais at three sites where SOD had killed most overstory tanoak and dramatically increased understory vegetation density and fuels. A separate experiment in an uninvaded, at risk forest was also conducted near Lacks Creek, in Humboldt County. The treatment was an attempt to increase forest resiliency to catastrophic loss of tanoak, increased fuels associated with tree mortality, and densification of the understory that are expected to accompany disease in these stands within the coming decade. Restoration experiments employed two types of mastication of understory vegetation and hand-crew thinning with pile burning; resiliency experiments employed hand crews. Treatments were compared to a set of reference conditions representing overly dense stands where intervention is needed and extensively managed stands that serve as management targets. Both restoration and resiliency treatments greatly reduced density of key sporulation supporting hosts with modest-to-minimal effects on stand basal area. The study found that prior land use, especially past harvesting, was a primary factor determining treatment costs and potential disease impacts in treated versus untreated stands. Although both restoration and resiliency management strategies were found to likely to reduce disease impacts, treatment costs vary substantially and greatly influence when and where each approach is optimal (Cobb, et al., 2017).

The project is in its fifth year and the District has funded monitoring work on the project through the USFS for another year. At the end of the 2019 field season, the research team will begin working on another publication, which is expected to be completed in 2020. The publication will provide the results of the five-year pilot study and associated monitoring. This study will provide the District with scientific information to justify and guide future management actions to increase resiliency to SOD, climate change, and other threats. The knowledge gained from ongoing SOD treatments will be incorporated into and will be implemented as part of the BFFIP.

Response to Comment A7-33

Broadcast burning is a technique that can reduce fuel loads and improve habitats, as the commenter states. The commenter questions why broadcast burning isn't a more important feature of the District's plan. Broadcast burning is, in fact, an important feature of the District's plan. Broadcast burning is proposed under MA-23 through MA-27, as shown in Table 2.9-1 of the Draft EIR. At full implementation, the plan includes up to two broadcast burn projects per year for forest-stand structure improvement (MA-23) and up to three projects for grassland and oak woodland improvement (MA-24).

The commenter notes that pile burning does not have the ecological benefits of broadcast burning, nor does flaming. The commenter is correct; however, the purpose of pile burning, and flaming, is to eliminate fuel loads cleared using mechanical and manual methods. It is not meant to be an ecological enhancement like broadcast burning. Pile burning and flaming are not "favored" over broadcast burning, as the commenter states, as these methods have very different functions from broadcast burning (namely, to eliminate fuel loads before manual or mechanical removal or after manual or mechanical removal).

The commenter requests the expansion of prescribed burns. The comment is noted but no changes have been made as prescribed burning is an important part of the plan already. As stated in Response to Comment A7-21, not every management action individually would achieve every goal of the BFFIP; rather, all the activities proposed in tandem would achieve the goals of the BFFIP.

Response to Comment A7-34

The comment is noted that invasive species removal is essential to protect habitats, wildlife, and biodiversity. The statements made by the commenter regarding the District's ability to keep up with spread of broom are generally factually correct.

The commenter asks how the BFFIP will correct failures in keeping up with broom spread to reduce the broom spread in the future. The goal to reduce broom spread is one of the primary goals of the plan. Several of the approaches identified to achieve the goal of enhancing biological resources in the BFFIP, address weed invasions as well. Invasive plant species would be targeted specifically under MA-22 and MA-24 but would also be treated under other management actions, including MA-20 (where weeds occur in existing fuelbreaks), MA-21 (where weeds are found in new fuelbreaks), and MA-22 (where new weed infestations can be treated with early detection), and rapid response (EDRR). MA-27 would involve implementation of weed control trials. Chapter 7 of the BFFIP identifies the goals of the plan and the increased resources that will be committed in the future to implementing the BFFIP.

Response to Comment A7-35

The commenter requests information on the conditions under which glyphosate use presents a risk to humans. Impacts of glyphosate on the public and applicators (humans) is discussed on pages 4-29 through 4-30 of the Draft EIR. The District's previous procedures for application of glyphosate were similar to those proposed under the Limited Use of Herbicides Alternative, including the numerous restrictions. The commenter asks for the District's procedures for using glyphosate when it was successfully treating and to compare them to conditions that pose a health risk. The risks at that time are the same as would be under current conditions, which again, are addressed on pages 4-20 through 4-30 of the Draft EIR. Refer to Master Response **6: Limited Use of Herbicides Alternative** for an additional discussion of the herbicide alternative and why it was not identified as the environmentally superior alternative.

Response to Comment A7-36

The commenter asks for how other jurisdictions use herbicides as part of broom control, the protocols of those agencies and the success of their efforts, and if other herbicides besides glyphosate are used. The intent of the comment appears to question the merits of the BFFIP as proposed without the use of herbicides. While other jurisdictions may use herbicides, it is beyond the scope of the CEQA analysis to address comments on the merits of the plan. The plan, as proposed and as is assessed under CEQA, does not include herbicides. The effectiveness of herbicides is not a CEQA consideration under the proposed plan. The alternatives analysis addresses herbicide use as an option and identifies the impacts of herbicide on District lands (see **Master Response 6: Limited Use of Herbicides Alternative**), but CEQA does not require the assessment of effects of not using herbicides.

Response to Comment A7-37

The commenter states that the District plans no action in areas of large broom infestations and that leaving these areas untreated will result in expansion of highly flammable broom, increased habitat loss, increased fire risk, and increased liabilities. The commenter is referring to the Deferred Action Zone. Refer to **Master Response 2: Ecosystem and Fuels Deferred Action Zone** for an explanation of the work that would occur in these areas. The strategy for this zone is to defer large-scale action but contain weeds where strategically possible. Maintenance activities occur in this zone under existing conditions and would continue to occur following implementation of the BFFIP. No change in the management of this zone would occur compared to existing conditions as a result of plan implementation. Any existing environmental effects associated with the maintenance activities in this zone are part of the baseline conditions for the EIR (CEQA Guidelines Section 15125). No new impacts would occur as a result of the BFFIP.

Response to Comment A7-38

The commenter asks about the District's liability should a fire erupt. The District's liability in the event of a wildfire is outside the scope of CEQA and the CEQA analysis. MA-10 includes attending monthly FIRESafe Marin meetings, which are also attended by landowners. MA-10 also includes supporting local fire departments in improving community education regarding defensible space, vegetation maintenance, and emergency response (as stated on page 5-3 of the March 2019 Draft BFFIP).

Response to Comment A7-39

The commenter asks if herbicides could be used under MA-27. No herbicides are included under the BFFIP management actions, as stated on pages 2-11 and 2-45 of the Draft EIR (and elsewhere). MA-27 is described on page 6-12 of the March 2019 Draft BFFIP. The management action includes grazing. The commenter also asks how an experimental method would be defined and if a method being used elsewhere would be considered experimental. The management action is open-ended, and the definition of methods would be up to the District staff but would generally be limited to small areas of less than 10 acres in size, including methods being used elsewhere but not by the District. These activities would be covered under the BFFIP and EIR as long as the impacts fall within those identified in the EIR. A Project Environmental Checklist form (Appendix A to the EIR) would be completed to determine whether or not the activity is covered under the EIR or if additional CEQA review is needed. Refer to **Master Response 7: Benefits of the BFFIP and Program EIR** for more information.

Response to Comment A7-40

The commenter states their opinion that the review of the proposed alternatives is flawed. The comment is noted. Chapter 4 of the Draft EIR identifies the increased, decreased, and unchanged environmental impacts of each alternative, including the benefits. Increased expansion of broom is not a significant impact of the plan, it is part of the baseline conditions.

Response to Comment A7-41

The commenter is correct in their summary of the refocused effort alternative. The commenter asks why the long-term impacts on habitat are likely to be greater under this alternative and why continuous removal of broom is considered a negative for this alternative. The Refocused Effort Alternative would refocus plan efforts from forestry actions to intensive treatment of weeds and habitat restoration in the areas most highly infested with broom and nearest to communities. This alternative is not as desirable as the proposed action because it would only address invasive species concerns. The management actions designed to enhance forest health would not occur as part of this alternative. Although more areas with broom infestations would be treated, areas impacted by SOD and encroachment of Douglas-fir would not be treated. Most of the broadcast burning for forest stand and oak woodlands included as part of the proposed plan would not occur.

The referenced statement by the commenter that “more areas overall could be treated under the proposed plan” pertains to the proposed plan, not the Refocused Effort Alternative. The areas treated under the Refocused Effort Alternative are summarized in Chapter 4, under Section 4.5.2 on pages 4-11 through 4-12 of the Draft EIR. Although the Refocused Effort Alternative would treat more areas heavily infested with broom, fewer locations and habitat types would be treated compared to the proposed plan. Increased treatment of broom is not in itself a negative, but in comparison to the proposed plan, the trade-off of increased treatment of broom compared to forest stand treatments would result in reduced benefits and effectiveness as compared with the proposed plan.

Response to Comment A7-42

The comment is noted that the commenter has no comments on the No Broadcast Burn Alternative. The No Broadcast Burning Alternative was identified as superior by eliminating the significant and unavoidable impacts on air quality and GHG emissions; however, the Draft EIR notes that the alternative does not meet all of the goals of the plan (page 4-31 of the Draft EIR).

Response to Comment A7-43

The commenter's summary of the Limited Use of Herbicides Alternative is accurate.

Response to Comment A7-44

The commenter asks for a discussion of the herbicide use of other jurisdictions, bringing the merits of the plan into question. Refer to the Response to Comment A7-36 for a discussion of the relevancy of the use of herbicides by other jurisdictions to the CEQA analysis in the BFFIP.

Response to Comment A7-45

The commenter asks if any other herbicides have been used to treat broom. The BFFIP does not include the use of any herbicides even though there are other herbicides available that can treat broom. The Limited Use of Herbicides Alternative includes use of other herbicides that could treat broom (triclopyr), which could have unknown health impacts, as stated in **Master Response 6: Limited Use of Herbicides Alternative**.

Response to Comment A7-46

The commenter asks how far in advance areas to be treated with herbicides (under the Limited Use of Herbicides Alternative) would be noticed. The duration of advance notice would be determined by District staff at the time of posting, given season, area, visitor-ship, and other factors to give people plenty of time to plan to avoid the areas under the Limited Use of Herbicides Alternative.

Response to Comment A7-47

The commenter states that application of herbicides near seasonal wetlands (under the Limited Use of Herbicides Alternative) should not be determined on a case-by-case basis and should be 100 feet. This change has not been made because, as stated, the stipulation would still require the District to create a buffer that is protective of the resource, which under these provisions may be greater or less than 100 feet. It should be noted that the stipulation includes Class III or IV streams and seasonal wetlands together in the requirement for a case-by-case consideration of buffer distance.

Response to Comment A7-48

The concern by the commenter regarding impacts to nesting birds from the **Limited Use of Herbicides Alternative** is addressed later in the section on pages 4-28 through 4-29 of the Draft EIR and includes surveys and application of mitigation to reduce effects to birds to less than significant. Limited herbicide use could reduce the need for mechanical and manual methods, as the commenter states.

Response to Comment A7-49

The commenter's statement that the Limited Use of Herbicides as part of IPM would meet the goals and objective of the program is noted, as is their disagreement with the conclusion that the use of herbicides would bring increased risks to human health, habitat, and water quality. Refer to **Master Response 6: Limited Use of Herbicides Alternative** for a discussion of the herbicide alternative and why it was not identified as the environmentally superior alternative. Impacts of glyphosate on the public and applicators is discussed on pages 4-29 through 4-30 of the Draft EIR. The impacts are identified in this section of the Draft EIR as likely less than significant, but there generally is a lack of consensus in the scientific community on impacts of herbicides on health. These risks to health do not occur under the proposed plan.

Response to Comment A7-50

The commenter's strong disagreement that the Limited Use of Herbicides received "minimal community acceptance" is noted. Refer to **Master Response 6: Limited Use of Herbicides Alternative.** While the Draft EIR identifies that the use of herbicides had limited community acceptance, the alternative was not identified as the environmentally superior because it did not address the significant and unavoidable air quality and GHG impacts of the BFFIP (from prescribed burning), and it could introduce new impacts related to health hazards, the extent of which is likely less than significant but not definitively known. The BFFIP is designed to reduce the impacts of invasive species and improve ecological health on the watershed, even without herbicides. The plan includes criteria and goals to be addressed annually by the District's Board. The adaptive management aspect of the program will allow for reassessment of methodology, noting that herbicides would not be included without further environmental review and Board approval.

Response to Comment A7-51

The commenter's opinion regarding the need to revise the impact discussions and the plan's impact on wildlife and biodiversity is noted. Minor revisions have been made to the Final EIR to clarify and bolster some of the existing mitigation measures, as shown in Chapter 3 of this Final EIR. No new significant impacts that were not already addressed in the Draft EIR were identified, nor were any new mitigation measures required after review of the comments received.

2.4 RESPONSES TO INDIVIDUAL COMMENTS

From: Bill Rothman <w1rothman@gmail.com>
Sent: Tuesday, April 9, 2019 8:02 PM
To: Biodiversity, Fire and Fuels Integrated Plan EIR
Subject: input for BFFIP

Comment Letter B1

From William Rothman, MD
14 Cliff Road
Belvedere, Ca 94920
415-435-1096

I am writing in support of the BFFIP plan, particularly the aspect of the plan that preludes the use of chemical pesticides, including herbicides. B1-1

Please acknowledge receipt of the input.

Sincerely,
William Rothman

2.4.1 Letter B1: Bill Rothman

Response to Comment B1-1

The support for the BFFIP as proposed, without the use of herbicides, is noted.

Comment Letter B2

From: Toni Shroyer <tonishroyer@hotmail.com>
Sent: Wednesday, April 10, 2019 2:39 PM
To: Biodiversity, Fire and Fuels Integrated Plan EIR
Cc: Larry Bragman
Subject: For your consideration: Please support protecting our watershed from toxic herbicides

Dear Shaun Horne,

I support protecting our watershed, and our water source, from toxic herbicides.

My family does not use roundup or any other toxins on our properties---- and we hope MMWD will follow suit and not hurt our environment. B2-1

Thank you,

Toni Shroyer
415-640-2754

Toni Shroyer Realtor, SRES (Senior Real Estate Specialist)

Coldwell Banker Residential Brokerage
2018 #1 Producer Novato Office Bradley Real Estate and Company-wide
2017, 2015 # 1 TOP PRODUCER SAN RAFAEL OFFICE For Bradley Real Estate
2018, 2017, 2016, 2015 Chairman's Club For Bradley Real Estate
2014 President's Club For Bradley Real Estate
TAN--- TOP AGENT NETWORK
415-640-2754
ToniShroyer@hotmail.com
www.tonishroyer.com
DRE #01876201

2.4.2 Letter B2: Toni Shroyer

Response to Comment B2-1

The support for the BFFIP as proposed, without the use of herbicides, is noted.

From: martine algier <martinealgier@gmail.com>
Sent: Friday, April 12, 2019 11:03 AM
To: Biodiversity, Fire and Fuels Integrated Plan EIR
Subject: glyphosate etc. / S996/BFFIP

Comment Letter B3

Our watershed is so precious
Please protect it.
The data is in.
We know these things poison all living beings.
Your grandchildren will thank you.
Please protect the watershed.
Gratefully,
Martine

B3-1

2.4.3 Letter B3: Martine Algier

Response to Comment B3-1

The support for the BFFIP as proposed, without the use of herbicides, is noted.

From: Dora Howard <beachbumpv@yahoo.com>
Sent: Saturday, May 4, 2019 1:12 PM
To: Biodiversity, Fire and Fuels Integrated Plan EIR
Subject: Fuel reduction process/decision

Comment Letter B4

Hello,

As a resident of the Deer Park neighborhood in Fairfax, I am extremely concerned about the possibility of a wild fire exacerbated by heavy fuel loads. To help reduce or mitigate the potential, I support a program using goats to clear some of the load. I'm aware that goats have been successfully used in other parts of Marin, such as Sleepy Hollow, and thus support their use in the Deer Park area as well.

B4-1

I look forward to receiving notification of this critically important decision.

Thank you,

Dora Howard
21 Spring Lane

2.4.4 Letter B4: Dora Howard

Response to Comment B4-1

One of the goals of the BFFIP is to minimize risk of catastrophic wildfires. Fuelbreak and WAFRZ maintenance and construction are some of the techniques proposed as part of the BFFIP to minimize wildfire risk. Grazing is included as a tool that can be used under the BFFIP. Refer to **Master Response 5: Grazing** for more information about grazing as a tool for vegetation management under the BFFIP.

Comment Letter B5

From: Mia Pritts <mia.pritts@gmail.com>
Sent: Monday, May 13, 2019 10:12 PM
To: Biodiversity, Fire and Fuels Integrated Plan EIR
Subject: fire fuel reduction - grazing in Deer Park and surrounding areas

Dear MMWD Board of Directors,

I live in the Deer Park neighborhood of Fairfax, which is beautiful and wild and overgrown to a point that is very, very concerning to me as a resident - and especially as a resident who lives in the back corner of the neighborhood, and for whom evacuation would be VERY challenging in the event of a wildfire.

I am writing to encourage you to bring goats or other grazing animals to our area and other areas of Marin, to help broaden the effort and effect of fire mitigation efforts. We all know that overgrowth is a huge hazard, and we've seen the price different communities have paid over the last several years of extreme fires. To be honest, I'm not sure there's any one thing we could do to really ensure containment of a fire under extremely windy conditions, but one thing I do know is that it makes sense to fund a variety of efforts to fight back the immense amount of growth and overgrowth here. B5-1

Our neighborhood is in the process of becoming FireWise certified and believe goats/grazing would certainly help as we work towards better preparation. I thank you in advance for your kind attention to my request, and am happy to answer any questions you may have about my community.

Best,
Mia Pritts
198 Meernaa Ave.
Fairfax

2.4.5 Letter B5: Mia Pritts

Response to Comment B5-1

One of the goals of the BFFIP is to minimize risk of catastrophic wildfires. Refer to **Master Response 1: Definition and Location of New and Expanded Fuelbreaks** for descriptions of the fuel load reductions proposed as part of the BFFIP. Fuelbreak and WAFRZ maintenance and construction are some of the techniques proposed as part of the BFFIP to minimize wildfire risk. Several of the management actions proposed as part of the BFFIP would reduce fuel loads, which can reduce the spread and intensity of a wildfire should one ignite.

Grazing is included as a tool that can be used under the BFFIP. Refer to **Master Response 5: Grazing** for more information about grazing as a tool for vegetation management under the BFFIP.

Comment Letter B6

From:	Roger
To:	Shaun Horne
Cc:	Ben Horenstein
Subject:	BFFIP and the BFFIP EIR
Date:	Wednesday, May 29, 2019 3:23:25 PM

Good afternoon, Shaun and Ben : I have had a look at the BFFIP and the accompanying EIR and conclude that these documents are basically are Birdseye view of MMWD's Vegetation Management Plan going forward on a Programatic basis . They do not appear to delve into specific action plans which will actually be developed in the future as part and parcel of an Annual Planning Process. It appears that it is the annual work plans that will lay out the proposed specific vegetation management and implementation project plans and priorities for the coming year.

Consequently, I would like suggest that it is going to be important that the BFFIP annual project plans include a careful public process not only with respect to the proposed project planning selections but also for their associated Environmental impact review . I, therefore, would like to propose that it would be worthwhile to spell out this review process now in advance as part of this current BFFIP and EIR review process. B6-1

Thank you for your consideration of this proposal.

Roger Roberts
223 Southern Heights Blvd.
San Rafael, Calif. 94901

Tel: (415) 457-7450
E Mail: <rer.dlr@ comcast.net>

2.4.6 Letter B6: Roger Roberts

Response to Comment B6-1

The BFFIP is intended to supersede the 1995 Vegetation Management Plan (VMP), which the District is currently implementing on its lands. The BFFIP describes vegetation management actions that the District would take over many years to minimize fire hazards and improve ecological health on District lands. Performance criteria for each management action are identified in Table 2.7-1 and were analyzed in the Program EIR. Activities to be completed each year would be presented in an Annual Work Plan. The activities proposed each year may vary in focus, depending on outcomes from previous years.

For future actions, the lead agency must evaluate and document whether the activity is within the scope of the Program EIR (Section 15168(4)). A Project Environmental Analysis form has been developed to assist the District in determining whether the activities proposed in each of the Annual Work Plans is within the scope of the Program EIR. Appendix A details the flowchart and steps the District would take to evaluate the Annual Work Plans for their coverage under the Program EIR.

Refer to **Master Response 7: Benefits of a Programmatic EIR** for additional information on how vegetation management would be undertaken in the absence of an approved BFFIP and Program EIR and the advantages of having an approved BFFIP and Program EIR.

Comment Letter B7

From: Ruth Todd <hrtodd@gmail.com>
Sent: Tuesday, June 11, 2019 8:37 PM
To: Biodiversity, Fire and Fuels Integrated Plan EIR
Subject: In support of goat grazing as a fire control measure in Deer Park et al

I understand that goat grazing in Deer Park is being considered in the EIR and I support this idea. Would prefer that the goats be temporary residents of the park and not year-round, if that is possible. B7-1

Thank you,

Ruth Todd
113 Hillside Drive, Fairfax

2.4.7 Letter B7: Ruth Todd

Response to Comment B7-1

The comment is noted regarding the residency of goats. Grazing is included as a tool that can be used under the BFFIP. Refer to **Master Response 5: Grazing** for more information about grazing as a tool for vegetation management under the BFFIP.

From:	Georgia Gibbs <hello@georgia-gibbs.com>	Comment Letter B8
Sent:	Wednesday, June 12, 2019 2:56 AM	
To:	Biodiversity, Fire and Fuels Integrated Plan EIR	
Subject:	introduction of grazing to reduce fire fuel in Deer Park	

Dear Shaun Horne, I am a resident of Deer Park and my home is less than 1/4 mile on 2 sides from the watershed and open space. I would like to add my support for grazing as one of the methods to be utilized in controlling fire fuel. I also feel it is important to protect the diversity of our natural habitat in support of the health and well being of all inhabitants–from small native pollinators to our wild creatures. I feel grazing could be an effective tool to accomplish both a safer area for human inhabitants and the other inhabitants we share this land with, keeping us all safer from fire. B8-1

Thank you for your time and consideration.

Sincerely, Georgia Gibbs

Georgia Gibbs Design & Fine Art

Fairfax AIR 2018 -2020

____

415.261.8928 | GEORGIA-GIBBS.COM | INTSTAGRAM

2.4.8 Letter B8: Georgia Gibbs

Response to Comment B8-1

One of the goals of the BFFIP is to minimize risk of catastrophic wildfires. Refer to **Master Response 1: Definition and Location of New and Expanded Fuelbreaks** for descriptions of the fuel load reductions proposed as part of the BFFIP. Fuelbreak and WAFRZ maintenance and construction are some of the techniques proposed as part of the BFFIP to minimize wildfire risk. Several of the management actions proposed as part of the BFFIP would reduce fuel loads, which can reduce the spread and intensity of a wildfire should one ignite.

Grazing is included as a tool that can be used under the BFFIP. Refer to **Master Response 5: Grazing** for more information about grazing as a tool for vegetation management under the BFFIP.

From: Christina Bertea <singingwater@jps.net>
Sent: Thursday, June 13, 2019 6:06 PM
To: Biodiversity, Fire and Fuels Integrated Plan EIR
Subject: Marin Water District No Herbicide Vegetation Management EIR

Comment Letter B9

Dear Shaun Horne, Natural Resources Manager: (bffipeir@marinwater.org)
I wish to write in strong support of Marin Municipal Water District's Biodiversity, Fire, and Fuels Reduction Integrated Plan.
I understand the comment period has been extended to June 19, 2019 and that this comment is well within that deadline. B9-1

I particularly applaud the decision NOT to use herbicides within the relevant lands.
I find myself often at events/conferences where the dangers of glyphosate—i.e. RoundUp (in particular) are being examined.

Here is my understanding:
Glyphosate was first developed as an antibiotic, which means it kills microbes.
In human guts I am told it is harmful to the beneficial gut bacteria, selecting for the less desireable ones.
In the soil it disrupts the microbial communities that are so essential to the cycling of nutrients to/among the trees and native plants.
And essential to the holding of water in the landscape!

The ever advancing study of soil microbes is revealing that certain types of fungus, in particular, sequester carbon in very stable forms in the soil.
(Glomalin and humus)
That is good news for our climate chaos dilemma.
But even more impactful, for every 1% increase of carbon in the soil an acre of soil is able to hold between 17,000 and 25,000 more gallons of water!!! B9-2
Soil microbes also participate in the aggregation of soil particles, leaving air spaces which allow the rapid absorption of rain, and eventual infiltration
of stormwater into aquifers. This is fondly referred to as the "soil carbon sponge".
These functions should be of primary concern in a landscape designed to provide water for an ever growing population.

We would do well not to disrupt these microbially generated ecosystem services by applying herbicides, as we search for ways to control invasive plants.

It may seem appealing to apply magical chemicals to kill invasive weeds, and there is no denying the degradation of the landscape that those weeds bring.
But the unintended consequences to human health and soil microbial health are not worth it!

The water district is tasked with providing water of optimal quality—and glyphosate contaminated water would not meet that criteria, irregardless
of what the bought and paid for EPA says!!!

May the refusal to use herbicides on water district lands stand firm!
Thank you,
Christina Bertea
water activist and eco artist

1

2.4.9 Letter B9: Christina Bertea

Response to Comment B9-1

The support for the BFFIP as proposed is noted.

Response to Comment B9-2

The comments regarding glyphosate are noted. Use of herbicides is not included in the BFFIP. Refer to **Master Response 6: Limited Use of Herbicides Alternative** for more information.

Comment Letter B10

From: lito brindle <litobrindle@hotmail.com>
Sent: Tuesday, June 18, 2019 7:45 PM
To: Biodiversity, Fire and Fuels Integrated Plan EIR
Subject: public comments
Attachments: BFFIP comments.docx

Enjoy!

Sent from Mail for Windows 10

1

1

BFFIP comments

Towards A Tamalpais Almanac

"These cats that I be hangin' around don't listen."

-E40

First I should state that I'm an employee, and I've worked on the MMWD watershed since 2004. I'm a maintenance worker, so I'm one of the ones in the field, all day e'ry day, implementing the sometimes inscrutable directives of plans like these. It is a peculiarity of such plans that they are almost invariably authored by the becubicled, and the deskbound. Sometimes as the proud industrial athletes of the watershed maintenance crew are returning from the grade in the afternoon and we see the managers through the windows right where we left them in the morning, peering at LIDAR images and spreadsheets and so forth we joke that our headquarters, Sky Oaks, might be mistaken for a minimum security sanatorium for the treatment of Nature Deficit Disorder – among many other things – the subject of a treatise of mine entitled "Last Manager In The Woods: Saving Our Natural Resource Management Professionals From Nature Deficit Disorder."

Also, I'm a third-generation rate-payer, and when I parse MMWD's mission statement – "To manage our natural resources in a sustainable manner, and to provide our customers with reliable, high quality water at a reasonable price" -- I think back and marvel that I don't EVER remember a time that reliable, high quality water didn't issue from the pipes 24/7, an outright miracle that it's easy to take for granted. I just barely remember the drought years of the 70's, when all throughout the service area the hot tubs operated at an average 11% capacity – it was a real bummer, hard times indeed for "macramé Marin" (and you thought YOU had experienced adversity) – and even then the excellent water never stopped coming out of the faucet. Visiting my grandparents in Willits I would marvel that the water would taste and smell different depending on when I visited, and that my grandmother would sometimes boil it on the stove on the advice of the municipal provider before giving it to us to drink! We had to make our own emojis back then, and observing that left my child's face the very picture of saddened confusion. B10-1

The FIRST half of MMWD's mission statement, though, reads: "To manage our natural resources in a sustainable manner…." It's posted prominently in several places around our headquarters, and at a not quite conscious level I would say I have been proud since I started as a seasonal on the watershed back in 2004 to work for an organization that aspires to manage its natural resources in a sustainable manner. But it's one of those things that dawns upon one gradually then suddenly, and after about fifteen years engaged in the work of managing our natural resources I've asked some of my colleagues this question and been greeted with crickets: Can anyone at MMWD name me even ONE way in which we manage our natural resources in a sustainable manner? The following are some observations about ways in which we most patently DO NOT, and some thoughts about how we might, going forward. B10-1

First, I think we can all agree: The roads and freeways are California's tragic commons. "The tragedy of the commons" is a concept known to every lower-division student of urban planning, environmental sciences, etc. It was explained to me by our own aquatic ecologist – fittingly, as we drove many miles downstream in a truck to grope special status smolts – and Wikipedia sums it up beautifully: "(A) situation in a shared resource system where individual users acting independently according to their own self-interest behave contrary to the common good of all users by depleting or spoiling that resource through their collective action." It was first pointed out where common grazing lands quickly became overgrazed. California's tragic commons is a trillion dollar plus constructed one, a million acres of asphalt, concrete and steel, perpetually stampeded and jammed up by lethal beasts carrying a purportedly intelligent species around in their bellies.

I read most of the BFFIP, and I tried to read the phone book thick EIR, but I'm a working person in urban California and I'm right at the end of my wits, such as they are. Who has the Time, Strength, Cash or Patience? I did get far enough along in the executive summary of the EIR, however, to read the incredible contention that the car trips that the BFFIP will set in motion are "not significant" because they generate "less than 100 peak time car trips." To me, that is a patently inadequate, arbitrary, and ridiculous standard. Tell that to anyone at all who has tried to get anywhere at all on California's tragic commons in our greater megalopolis any time in the last 30 years. Hopefully there are charismatic professors of urban planning, environmental sciences, etc. somewhere encouraging their students to rip that page out of the document it was originally published in, and the EIR's into which it was cut and pasted (perhaps this very one?), a la Dead Poets Society. I think more and more people would agree with me that the just about the WORST thing that you can do nowadays as a corporate citizen in a coastal megalopolis is to set vehicle trips in motion, and that EVERY corporate citizen should be thinking hard about EVERY such trip that they do set in motion. My greatest quibble with this plan is that it seems to consider such trips not at all. Consider that we've been working off the previous veg. plan since 1994 and you'll see that the proposed updated BFFIP is a prescription for literally innumerable car trips. B10-2

How about if we were to lead the way and OWN those vehicle trips that we are responsible for? A good way we could do that would be to compile and publish a commute map, and to update it annually. I believe every employer, or every commercial address, in every beclustered megalopolis ought to have such a map posted prominently in its lobby and on its website. Such a map would help any given organization, whether they consider themselves keepers of the public trust (as I think we do) or not, to be cognizant of the fact that, every action that we take, every working day, comes with these vehicle trips attached. Transportation, I believe, is California's greatest contributor of greenhouse gasses. The quality of the air, and the water, and the climate die a death by a thousand cuts – or rather, 7 or 8 *billion* cuts – every day, and I would argue that the air and the water and the climate are included in the "our natural resources" in our mission statement, because those are everyone's responsibility. So let's

3

carefully consider our own cuts. I was privileged to take a GIS class from Nick Salcedo, College of Marin's great professor emeritus of Geography. The very first lesson of his course was that depicting a problem graphically is sometimes essential to getting our species to perceive it, and hence care to solve it. In that case it had to do with the incidence of cholera, or dysentery, or something like that, in medieval London, or someplace like that (you can see that I was an indifferent student. It's not the great professor emeritus' fault. He was as attentive and diligent with me as The Miracle Worker with Helen Keller, but he couldn't have known that I'm a working person in urban California and I'm right at the end of my wits, such as they are, and "You can't get blood from a turnip" is more an epiphany from the Humanities). When those early berobed medieval planners and public health officials mapped the incidence of whatever the disease was around their city, and then mapped the public wells, and then the latrines, they noticed a co-incidence, and hence that great urban planning breakthrough: Maybe we shouldn't put the latrines and the wells right next to each other? They were ALL awarded PhDs for that original contribution to human knowledge. Seems obvious in retrospect, but who knows but that without the map we wouldn't still be defecating in the wells? It's a water quality parable, after all.

But I firmly believe that the "commute," as it's currently understood and practiced, will be as incredible to future generations as such practices of our not so distant antecedents of dumping raw sewage in The Bay, burning and burying our own trash, hunting to the ends of the earth and slaughtering whales *to light our lamps*, etc. So let's be a leader and OWN the vehicle trips that we solicit, that we are responsible for, that we deem necessary and worthwhile to accomplish our natural resource management objectives, and then we can begin discussing whether or not they gibe with our published aspiration "to manage our natural resources in a sustainable manner." Let's request such a map of our contractors, too. I remember that the heavy equipment operators in one of our first SOD plots in the middle of nowhere Bolinas Ridge came from Sebastopol every day. Another contractor crew was rumored to be headquartered in Santa Rosa. I talked to some of the guys on yet another crew and they pile in the crew cap pickup in Richmond, but first they have to get to Richmond, and two of the places they mentioned coming from were Vacaville and Hayward. We've had seasonals coming from as far away as Concord, Castro Valley, Woodside, etc. And that's just us, away back at the end of the Ross Valley. Imagine the map if it included everyone on the Bolinas Road from Grilly's to the Meadow Club, or everyone on Nellen from Joann Fabric down to the Corte Madera Town Center. Extrapolate that map across the entire megalopolis and you have a spaghetti Rohrsach test that would be universally interpreted as poor urban planning. One day ABAG or someone like that should have such a map for every employer in the region, so let's be the first. Our watershed managers published somewhere that they were engaged in the "filling of data gaps to enable managers to make informed decisions." It used to be on our website somewhere, maybe since taken down. But if you really aspire to manage your natural resources in a sustainable manner, I would think that a commute map would be one of the first data gaps you would want to fill, long before you map and graph the peregrinations of the pallid fruit bat, etc. B10-2

And about those data gaps. I'm not sure it is properly MMWD's responsibility to fill perceived data gaps. I think mostly we do so out of an impulse to prosecute the scientific inquiry learned in the academic environment. Since I've been here we've even sponsored and organized more than one "symposium" B10-3 to share the scientific breakthroughs we've achieved, or rather the accretion to the body of scientific knowledge, and you can't help but come away from those with the impression that they are much more about being admired by scientist peers than they are about managing our particular resources in a sustainable manner. They are convened to flatter our own sense of our own efficacy at least as much as they are to share better methods of managing natural resources. They are a re-creation of the stimulating university environment, and tellingly, favorite professors are always invited. But our mission is not "Publish or perish." Can we name a single instance in which the filling of data gaps informed our stewardship, or we took home anything from any symposium that significantly helped us to manage our natural resources in a sustainable manner? Alice Eastwood is often held up as a paragon for the managers and professors at these symposia, but Alice Eastwood was famously in the employ of the California Academy of Sciences -- not, in other words, a humble utility with some lands to manage.

I would say it is more properly the responsibility of such organizations -- academies of sciences, the spectacularly well funded public research universities, etc. -- to fill those data gaps and hash out the state of the art BMP's than it is ours. It's up to us to *implement* those BMP's. If you hired an engineer to build you a dam, for example, and they told you, "I'm going to engage in engineering experiments for the rest of my time in your employment to sort of shore up my engineering game, but rest assured that the dam I design you at the end of that time will be state of the art!", I think you'd quickly find yourself another engineer. When we commit to "doing science" we quickly get into the weeds, as it were. We fail to see the trees for the forestry, or something like that. Paralysis through analysis. All of the "monitoring" prescribed by this plan is basically a prescription for endless botanizing picnics, a benefit perhaps to posterity but not to ourselves. With vast lands and limited resources, the prosecuting of scientific inquiry should not be our purview. We've been engaged in endless science projects since I got here, and the result is that much of our lands still look godawful. Had we put all of that energy into weeding and thinning, I think we'd have achieved better results by now. B10-3

In a phrase, we ought to direct our energies into, "More stewardship and less science." In fact our entire vegetation management plan could read: "Weed and thin (in a sustainable manner)." If we weed and thin in a sustainable manner, the plant communities will sort themselves out as they have always done. We've identified several areas in this plan for "restoration," but it feels like we're already fighting on way too many fronts, and the question is: "Restoration" to what? The notion of how to go about that changes, sometimes spectacularly. For instance, I believe in our previous plan it stated that we should "reveg." only with plants that are genetically appropriate to the particular sub-watershed being "restored." In the current plan we say that we will reveg with plants from "hotter and drier" areas of the watershed in the face of climate change, which is at best a guess. I'm sure that the authors of each were absolutely convinced of their ecological correctness and scientific rectitude. But that's a big 180, and not the first time we've reversed ourselves. We've been "restoring" Pine Point since I got here, and it still kinda looks like a trainwreck. Recently we spent a lot of time and effort taking out many of its namesake pines – which a previous generation of foresters *planted*, according to then state of the art forestry BMP's. A few years back we spent a lot of time and effort removing tires from Phoenix Lake which a previous generation of managers had *installed* there as "fish habitat." I would ask for an accounting of how it went with the Baker's Larkspur before I would green light the "restoration" of any particular rare plants or plant communities. Etc. If we commit to "restoring," cherished sites or species, I think we risk the certainty that a bully like the Tubbs or the Camp fires – or the Godzilla of climate change right behind them – is going to come along and stomp our meticulously crafted and frightfully expensive little sandcastle flat. Years ago we went around and monitored and mapped the species of special concern du jour, Napa False Indigo, including boat surveys on some of the reservoirs. A few years after that somebody went out ahead of some maintenance work along one of our roads to flag the Napa False Indigo so that the heavy equipment and trucks would spare it, and they started out flagging individual plants but then started stretching the flagging from plant to plant to save time. It looked like a mardi gras parade had passed. It highlighted the fact that the SOD tanoak dieoff has opened up the canopy and apparently actually proved a boon for Napa False Indigo, probably among many other species. Could you find the silver bullet to reverse SOD and restore the tanoak canopy, special status Napa False Indigo would likely suffer. I'm not sure how any of that monitoring in the meantime helped us, if you consider our mission.

The land, the plants – *life* – is resilient. The impulse to *do* something comes out of that old Garden of Eden anxiety, which reads, more or less: OMG, we're wrecking a perfect Creation. Or, a perfect Evolution, depending on your beliefs. It is an anxiety as old as the species. We felt it as we watched the La Brean megafauna die off, and understood our culpability. But it *be's* like that in the Anthropocene. New populations have always been invading, crashing, stabilizing, etc. Maybe before the mechanism of introduction was continental drift or meteors or siroccos and jet streams or the proverbial African swallow but so long as there are 8 billion of us propelled by fossil fuels ricocheting and pinging all around the region and all around the planet *all the time* the mechanism of introduction is going to be accelerated, and it's going to be us, every time. You can delay the introduction, but you probably can't stop it. Populations are going to crash, especially with so many of *us* blundering about. Garden of Eden anxiety has been with us since long before the story was written down, and accounts for that story's resonance ever since. It still drives us now, and irrupts in these prescriptions. But if we can just calm our ass down and weed and thin in a sustainable manner I think evolution can proceed and the plant communities can sort themselves out without our anxious ministrations as they always have. B10-3

So, how to weed and thin in a sustainable manner? I got some ideas…. Even after fifteen years of matching wits with French broom, the scope of the problem wasn't really brought home to me until I was walking my dog along a sidewalk next to a small broom stand during a heat wave and I saw where all the little glittering black broom seeds had popped onto the sidewalk and been tumbled by the wind into the "control joints," the grooves pressed into the concrete when it is placed to allow for cracking. You hear different figures but the one I hear most often is that those broom seeds can lay there and remain viable in the wild for *forty years*! When you consider that a single mature broom plant can produce, I believe, thousands of seeds per season, and that we have many thousands if not hundreds of thousands of broom plants out there on our lands already, you begin to dimly perceive the scope of the task. A few months ago I was stopping traffic on Shaver Grade at the middle Elliott Trail intersection as a fourteen ton excavator slowly mowed up the grade below me, and turning through 360 degrees I saw nothing but a wall of mature French broom up over my head. Then I reflected that during my first season in 2004 we spent much of our time cutting and pulling and spraying broom with Roundup, including all throughout this area – sometimes the lids or the pumps to pressurize the backpack sprayers would leak, and we'd wind up with our coveralls soaked with Roundup and the mixed Roundup with its blue indicator dye soaked down our backs and a free big blue tattoo -- and the broom's all back now worse than before. And now the entire management staff that was making the decisions in 2004 has aged out of the system and retired, political realities put an end to the Roundup, and here we are in 2019 and there's a contract crew pulling broom just up the road, which just seems arbitrary and futile when you take the long view. And ever since my first season, and of course long before, every day the car trips come from all around The Bay and return home after an arbitrary duration of stewardship. B10-3

The demographic that's likely to read the public comments of a plan such as this has likely enjoyed the privilege of trying, in vain, to stay ahead of the weeds in a postage stamp suburban backyard. Multiply that postage stamp by 100,000, conservatively, and you'll get an idea of what we're up against. But if we want the approved plants to grow, and the invaders to be extirpated, we're going to have to tend our lands like a garden – *forever*. Tending the wild (be sure to watch the excellent documentary by that name, available on the internet, that features indigenous Californians demonstrating how they did – and still do – tend their lands) is not a grant-funded initiative (ask about MMWD's ill-conceived "Broom-free by 2003!" campaign), it's not a volunteer picnic, it's not something that can be approached according to budget cycles and shifting management and political priorities. Tending the wild is a *role*, something we have to commit to doing every day so long as we are responsible for these lands.

If we are committed to that, it would seem that we *must* find actually sustainable ways of doing so. Foremost among those would be committing to shrinking – or better yet *outright eliminating* – our commute map. If you really want to eliminate just French broom, for example, you're going to have to put stewards out there for the next forty years….at *LEAST!* I'm talking about a network out on the watershed of "static stewards," quartered and provisioned after the spartan fashion of the backcountry rangers of the high Sierra. If they can manage the logistics of quartering and provisioning resident stewards in the roadless high Sierra, we can certainly do so in our heavily visited and travelled watershed near the teeming heart of a coastal megalopolis. Subtract the fossil fuels and the electricity and the amplification and the mechanized transportation and humans are almost not obnoxious out in the landscape. If your natural resources lie near the teeming heart of a megalopolis you can't have the people providing the stewardship laying their heads on the far margins of the megalopolis each night, at least not if you want to claim that you aspire to sustainability. So, pick a broomy pilot polygon or polygons and place static stewards out there – in tents, in yurts, in cabins, in treehouses, in all of the above. Such an experiment would doubtless attract enthusiastic design talent, when it comes to individual stewards' quarters. One good pilot site would be somewhere out past the Sack Shack at Sky Oaks. Could one or more static stewards get all the broom (embarrassingly encroaching right up to the doorstep of our headquarters), and provide substantial thinning, in the polygon bounded by the Sky Oaks Road, the Taylor Trail, Concrete Pipe Fire Road and Rattlesnake Creek? Pine Point itself would be another good pilot site. Or pick a pilot polygon in the dark, lawless heart of Broomlandia, amongst the worst scum of the deferred action areas – somewhere around Phoenix Lake or Deer Park – and see whether a static steward can't get all the broom in the polygon a season, and also provide thinning. Staff it for forty years and see if that does the trick. Broomlandia especially is only a short bike ride or B10-4 long hike from civilization, if you find that you miss it. With composting toilets and the means of treating their own drinking water, static stewards could probably stay out there in our mild coastal climate year round. In the way that the static, resident stewards of the Farallones are supplied and supported by a volunteer navy of pleasure boaters, perhaps a volunteer brigade of mountain bikers could deliver our stewards their peck of gruel and mail, etc. The entire watershed could be broken up into polygons, each of which a single static steward could maintain. Out in the relatively pristine areas like Big Carson the polygons would be vast, in the more compromised deferred action areas of Broomlandia they'd be smaller and denser. Experience would fine-tune what that map would look like. But giving individuals ownership of a specific geography seems like it would be more effective than following around the changing priorities of new management, new political realities, scientific fashions, plan updates, etc. Let's put the workers where the work is, and keep them there. Regionally, we designed and built all this superfluous movement into our system, at enormous expense and effort. In the face of climate change and unprecedented population pressure and density, it's time to design and build it back out. Let's design for Global Calming, again.

Who might staff such a spartan network of static stewards? The list is long…..

The newly graduated: It's probably not unique to Marin, but it's certainly a fact that when you grow up in Marin's strange cultural bubble, and you get to the end of high school, basically you know two things – how to complete homework assignments, and how to dress yourself. So what do we do? We send our kids off for four more years' – at *LEAST!* – practice completing homework assignments. And we tell kids when they have zero life experience and zero maturity, when not only do they not know anything at all about the world and about life but they don't even know anything about *themselves*: "Pick a career path." We end up squandering so much resources on kids when they are at their absolute most callow and hormonal and when they haven't any life experience to measure the abstractions of a higher education against. A gap season or year off or just barely on the grid could only do such lost kids good. Ditto for those newly graduated from college, or from graduate school. There's a lot of talk about a national service program for young people. A static stewards program at MMWD could be a shovel ready option, maybe with an educational stipend available at the end, with the millions we're apparently prepared to spend to manage our weeds and fuels problems. A season out on the watershed could undo a lifetime of Nature Deficit Disorder, for the youngbloods and for managers alike.

B10-4

The newly arrived: The best veg. management work we get is already provided by immigrants. When you happen to be working near those contract crews you will hear them keeping up a laughing patter all day and you will never hear a word of English, and they are apparently having a great time, all the while accomplishing incredible prodigies of grueling physical labor, sometimes on terrain that would make a goat curl into the fetal position. One can stand off at a little distance and watch them literally work circles around Bachelors and Masters of Sciences. With the cost of living in urban, coastal California, particularly in our Bay Area ground zero, it is, let's face it, only the privileged who have the luxury of volunteering, and if your volunteer program depends upon Marin students and parents shepherded by Bachelors and Masters of Sciences an affluenza outbreak could shut down your entire program at any

8

time. Give *us* your huddled masses, etc., in flight from war, poverty, etc. We can be a sanctuary watershed. Offering a meaningful thing like spartan accommodations and perhaps a relocation stipend might make such a relationship a win-win. And, in point of fact, MMWD *has* hosted such resident static stewards before, albeit unwittingly. I missed the excitement when our staff got to go in and take out the illicit marijuana plantations on our side of Bolinas Ridge circa 2006, but they were described to me as "vineyards," and the consensus from the paraphernalia laying about the camps was that they were probably Mexican nationals. Those anonymous ones, none of whom were apprehended, incredibly hewed *vineyards* of marijuana out of the wilderness, entirely off the grid, entirely by hand, in our absolute most remote and wild area. But any time you have a group of indigenous people cultivating an indigenous herb artisanally and off the grid and entirely by hand you've *got* to send in the Caucasians with helicopters and automatic weapons, because you *can't* have that. You just can't. So that's what we did, but it was evident by the way the trees and chaparral had grown around and absorbed some of the wire they'd put up that they'd been there for *years*. A coworker joked at the time, "We should hire *those* guys," and why not? For every acre you keep free from broom, you can grow ten marijuana plants, or twenty, or whatever's reasonable. It's an opportunity for One Tam-branded Tammy Whammy. Marijuana aficionados (we know they occupy every level of the decision-making and bureaucratic hierarchy, don't we?) often believe that plant to be the great panacea anyways. Let's put it out there and see if it can't outcompete broom. We agonize too much about being "ecologically correct," when really, in the Anthropocene it is what it is, right? The ecology, I mean. We've tried everything else, let's try Tammy Whammy. Responsibly. But if you turned a crew of men from rural Latin America loose with nothing but machetes you'd be astonished and humbled at the amount of thinning they could accomplish. Swede George was a resident woodcutter too, long before the 1918 construction of the Alpine Dam Gravity System, when someone familiar with the construction effort wrote of the men cutting the tunnel through Pine Mountain to deliver the water by gravity to the Ross Valley, "The first crews…were all Swedes and Italians but they weren't making any progress so we had to bring in crews of Whites"(**!**). Bill Williams was a humble woodcutter who lived in the steep box canyon now bearing his name that debouches into Phoenix Lake, a fire gamers nightmare – until he changed his name to the catchier Billy Dee Williams and his career took off in blaxploitation films and of course the Stars Wars juggernaut. So resident stewards providing thinning would not be without precedent. They denuded the entire state, more or less, in the days of the axe and the ox. Let's welcome the newly arrived. B10-4

Climate refugees: Once that meant people in flight from inundated atolls, but suddenly we see that can mean people from as close to home as Coffey Park, Paradise, etc. Inevitably one day those climate refugees will be our own watershed neighbors and rate payers. FEMA is helping the parks around Paradise build the infrastructure to quarter the climate-displaced indefinitely after the Camp Fire….maybe they might help us build our static stewards network *before* the inevitable disaster, which in earthquake country is at any time only a heartbeat away.

Our own staff: Employee health and well-being could only benefit from the opportunity to sidestep out of the frantically churning human gyre, and from the manic frenetic world that fossil fuels have made. For 100,000 generations we went nowhere our feet couldn't take us, and now for five or six or so we go

9

ricocheting and pinging all around the region and all around the planet *all the time*. We can give our own staff a meaningful break from that madness...let's call it "glewardship," "glamping" plus "stewardship." Any such sabbatical, whether it be for a week or a month or a year, should obviously be at *full* pay and benefits. Our own elected Board retreats could be glewardship retreats, too, Bohemian Grove without the exclusivity. Our seasonals, if they've got nothing pressing, could spend their offseason – and their on one, for that matter – staffing our static stewards network. Etc. I'm talking about the entire MMWD staff, but the natural resource management staff especially should be strongly encouraged to take at least a week a year for a glewardship sabbatical and get out there and earn and savor, in the words of the great Aldo Leopold: "(T)he sense of husbandry. It is unknown to the outdoorsman who works for conservation with his vote rather than with his hands. It is realized only when some art of management is applied to land by some person of perception. That is to say, its enjoyment is reserved for landholders too poor to buy their sport, and land administrators with a sharp eye and an ecological mind."

Writers and artists in residence: Such folks need a sanctuary from the cost of living, and the peace and psychic space to work in. Our static stewards accommodations would have to be just on the grid enough for them to work and to be in communication, but they could do their work in peace in the mornings when the mind is fresh and weed and thin in the afternoons, or vice verse, and I guarantee that after the *next* 100 years of stewardship hosting such resident fellows MMWD will truly have A Tamalpais Almanac to show, and will be able to proudly point to its contribution to the *cultureshed*. And while we're on the subject, can One Tam or somebody designate a Poet Laureate of Tamalpais, and name the great Fairfax resident and avid Tam recreationalist Kay Ryan the first one? I might even finish Last Manager In The Woods... during such a residency....

B10-4

Researchers: If you're a graduate student or other researcher with a NRM project to complete, and you want to monitor plants, or map seeps, or map and graph the peregrinations of the pallid fruit bat, etc., why, if you'll just weed and thin for us, you can stay and work as long as you like.

Indians: The Coast Miwok, who did such an incredible job of managing these lands for at least 2,000 years, might like to have a laboratory where they could practice Traditional Ecological Knowledge, cultural burning, etc. (again, you gotta watch Tending The Wild).

Millenials: In a hearty environment of fresh air, sunshine and hard physical work......

Far from air conditioned environs and with their hands right down in the dirt.....

On a spare but hale diet of gruel and perhaps salt beef and maybe porridge....

Unplugged from and independent of their devices.....

OK, maybe not Millenials. Forget the Millenials.

10

Anyone at all: It's funny to think that there are "forest bathers" who think they can approximate themselves to some trees for part of a day and that that will get the anxious stink of the megalopolis off them. Try an immersion program. There are likely many souls who always wanted to try a Thoreau-like experiment, and we could provide one that such people could step right into. Etc. B10-4

The thing about these weeds and fuels is that they are constantly proliferating. We want to burn to control them because the natives did that so well, but that really would not seem to be practicable nowadays. I mean, it's *possible*, but it's frightfully expensive and labor- and fossil fuels-intensive to burn. And it's risky. So, we need something that is constantly *digesting* the constantly proliferating weeds and fuels. MMWD ought to investigate a biomass microgrid to do just that, dispose of the weeds and fuels cleanly by turning them into power. We are, after all, the biggest power user in the county. We stood ready to undertake the expense and effort of building a pilot desalination plant not long ago, and weeds and fuels are obviously less critical to our mission than water but the task is no less prodigious. An organization with the wherewithal to build a pilot desal plant can build a pilot biomass plant. A portable biomass processor or processors could move around the watershed to the abatement sites and connect to the microgrid there, or biomass could come into a centralized biomass processor via self-governing gravity cars on the biomass railroad, for example. Or both. It was in our 2014 5-year strategic plan to do just that – "Assess portable cogeneration technology for forest management on Mt. Tam watershed" -- but if it was ever assessed at all I never heard the outcome. As a corollary to "Weed and thin in a sustainable manner," a slogan vis-a-vis biomass could be "Feed the *maw.*" We can dispose of our fuels and weeds by turning them into power in a biomass power plant, a little more challenging but much less obnoxious in the long run, and much less inefficient, than burning them in piles. One of the greatest advantages of biomass, too, is that as it comes in, as you are weeding and thinning, you could *weigh* it, and hence get good, hard data about how much weeding and, in particular, *thinning* you are actually doing. Inevitably a Camp/Tubbs/Oakland Hills fire will come motoring across our lands one day, and billions of dollars in property damage are going to ensue and people are going to *die*. Attorneys for the plaintiffs are going to want to know: How much thinning did you do? Now all we have to tell them is that we burnt x amount of piles, which is ultimately fake data because a pile is ill-defined, or that we spent y amount of money, which, as anybody knows, has little to no correlation to the amount of actual work being done. Even if we don't add biomass into the mix, we should add the capacity for weighing the weeds and fuels we thin. It would be a good way to solicit and administer contracts, for one thing: Here's your polygon – take six-and-a-half tons out of it and we're good. The capacity to weigh the weeds and fuels we take out would also be a great motivator for the people conducting the work, and a way to quantify the thinning provided by, for example, our static stewards. We could pit one or more crews of contractors or our own staff against each other in friendly competition. How come Division Zulu brought in 40 more tons than Division Alpha last season? Can the proud industrial athletes of watershed maintenance bring in more than the flaccid veg. geeks? For our volunteer program, can Tam bring in more than Redwood? Can the seventh-graders bring in more than the eighth graders? Can the girls bring in more than the boys? Can the environmental sciences class pull its own weight in broom (in a mature broom stand, this should be no problem)? We haven't done anything to harness the incredible masochistic energy of the crossfit crowd, but a Biomass Challenge could pit gyms against one another and individual athletes against each other and against themselves. B10-5

11

Our fir abatement out along West Ridgecrest, for example, leaves the tree boles jackstrawed across the landscape in a most unnatural fashion. If they were diced up to manageable size, a cross fit volunteer strike team would like nothing more than such a challenge as to shoulder them and carry them up the hill to be weighed and converted to biomass, and to know that they humped 10 tons of biomass 100 vertical feet up the hill and helped accomplish some significant natural resource management objectives while toning hearts and lungs and glutes of steel. Etc. The Doug fir caber toss, into the *maw*. So, bring *weight* data (and perhaps volume data, those grape bins you see in the wine country springing to mind) online, even before biomass. With portable industrial and agricultural scales, smart yarder grapples, and the means of leverages, purchases, and mechanical advantages, also, we would finally have an adequate and accurate means of weighing yo mama. B10-5

Another way we could weed and thin more sustainably is to construct a connected contiguous network of grazed fuelbreaks. Pit a biological agency against the proliferation of fuels and weeds, instead of fossil fuels and human institutional ADHD. If you've borne with me thus far, try flying in Google Earth to the intersection of Shafter Grade and the Bolinas Ridge Fire Road. The BRFR is roughly the boundary between MMWD's lands and the lands of the GGNRA, I think. There's a barbwire fence running along the west side of the road, and it's the line of demarcation for two land management philosophies and practices. A few years back I was sent up there for weeks on end in the winter, with chainsaws and polesaws and a fullsize pickup, along with a heavy equipment operator and a 14-ton excavator with a tree-obliterating mechanical head on it, to touch up the fuelbreak on our side of the fence. On our side it was an unbroken canopy of coyote brush dominated coastal scrub, and after weeks of fossil fuel intensive work we had obliterated almost all of the scrub stand, leaving what looked like an Ewok village of woodrat nests constructed of piled up sticks. Meanwhile, in our comings and goings we made the acquaintance of the nth-generation husbandman across the fence. His last name, as I remember, was Giacomini. We only saw him up there once, tending his cows. But it's one of those things that dawns upon you gradually then suddenly, you can't help but look upon those grazed lands across the fence and think: "Now *that's* a fuelbreak." Fly there in Google Earth and you'll see what I mean, the difference is readily apparent from outer space. Or if you know the territory imagine yourself standing on the little league diamond in downtown Nicasio, looking in the direction of the church. It should bring home to you the fact that responsibly grazed lands look *great*, and much more fire safe than non-grazed lands. You could probably stand there on the Nicasio little league diamond looking in the direction of the church, and if a Tubbs or Camp fire was bearing down on you from that direction you wouldn't feel threatened. Subtract the grazers from that landscape and within 10 years I would guess, at the most, you would be looking at an unbroken canopy of coyote brush dominated chaparral, and if a Tubbs fire were bearing down on you it would be the last thing you'd ever see. Academia is obviously hostile to cows, but if you know the territory you know that here in the birthplace of the agricultural land trust we're kinda fond of our cows and husbandmen. And, the husbandman is out there upon the land, where meaningful epiphanies happen. Or, in the words of Aldo Leopold: "(T)he farmer may see in his cow-pasture what may not be vouchsafed to the scientist adventuring in the South Seas. Perception…cannot be purchased with either learned degrees or dollars…" Nth-generation husbandman Albert Straus of Straus Family Creamery, who truly seems to care about and be committed to and provide leadership in the area of sustainability, had the epiphany that higher and higher housing costs and the longer and longer commutes that they engender are antithetical to sustainability (In his B10-6

12

words: "We cannot be sustainable if our employees cannot live here, if they have to commute long distances from Santa Rosa, Rohnert Park, Petaluma, Vallejo.") that apparently never occurred to us, or that we have never expressed. I would suspect that many of the areas where we are now engaged in fossil fuels intensive "meadow protection" have grass at all because previous generations of dairy operators favored grass there, such as the dairy family whose operation occupied much of the land surrounding Bon Tempe Dam and gave that dam and lake its misnomer. Lately we've been protecting what grass remains around there from Douglas fir and coyote brush encroachment with incontinent diesel-spewing excavators and skidsteer mowers.

A grazed connected and contiguous network of fuelbreaks would be a win-win, maintenance for us and range for the critters, and another marketing opportunity for One Tam, perhaps with MALT and Cowgirl Creamery, Fuelbreak Farmstead Fromunda to give out at the fermentation festival. Or, native grazers could maintain those fuelbreaks, like tule elk, described by Richard Henry Dana on the Marin cliffs of the Golden Gate in the winter of 1835 thusly: "The tide leaving us, we came to anchor near the mouth of the bay, under a high and beautifully sloping hill, upon which hundreds and hundreds of red deer, and the stag, with his high branching antlers, were bounding about, looking at us for a moment, and then starting off, affrighted at the noises which we made for the purpose of seeing the variety of their beautiful attitudes and motions." That would be ecologically correct, but as I've said, it's probably a waste of energy to agonize about ecological correctness in the Anthropocene. I transected the Diablo range from San Jose to Turlock, over Mt. Hamilton, to visit a friend at Stanislaus State in Turlock decades ago, and I remember as I found my way through the extensive terra incognita east of Hamilton being pleasantly astonished at the sight of a herd of bison that some rancher had on his lands. Some of us have experienced the same astonishment driving past the Hearst Ranch down around San Simeon and double-taking on *zebras* gazing back at us from the verdant hills. We shouldn't hesitate, even in the face of the protestations of those who insist upon ecological correctness, to take management actions that astonish and delight people. Sciencey types are adorable. They believe that the "best" argument is the most logically and empirically and scientifically sound and unassailable one. You will never persuade them otherwise. The *best* argument though, in point of fact, is the one that persuades the most people. Such arguments are almost *always* emotional ones – why do you think the data-driven arguments about climate change can't seem to gain any traction – and for better or worse it is upon such arguments that, in the Anthropocene, the fate of the planet and all its little live things hangs. So let's not be afeard to take big, symbolic actions, to do things that stir and delight people, and let's not be cowed by the protestations of the puritanical sciencey types, the likes of whom banished the nth generation husbandmen from Drake's Estero, etc. B10-6

Also, let's not be cowed by the counsel of the shamans of risk and liability. The shamans of risk and liability will be perpetually rattling their gris-gris abacuses in the faces of the benevolent chieftains (I'm talking about our elected Board), and they should be judiciously listened to, but occasionally they should also be put in their place, lest they lead us astray from our mission and our very values. For instance, I wonder if the benevolent chieftains know that it is the policy of MMWD that whenever one of its employees gets in an accident, and that accident is subjectively determined to have caused greater than or equal to $2,000 in damage, that employee is subject to a drug and alcohol urine analysis, the type of unreasonable search that is explicitly proscribed by The Constitution and literally often adds insult to injury? Doubtless that was the brainchild of some shaman of risk and liability, who ought properly to have been gently but firmly put in their place by the benevolent elected chieftains. So basically, we require our employees to surrender significant civil liberties – Constitutionally guaranteed freedoms – if they want a paycheck. If they want to eat. It's a bad look, particularly for a municipal utility in a self-styled politically progressive geography. Similarly, doubtless those shamans are going to say: "We couldn't possibly put static stewards out there in tents, yurts, cabins, treehouses……because something *bad* might happen." It is up to the benevolent chieftains to put 'em in check, and perhaps to mollify them we might graze some of our fuelbreaks with those goats that faint when you startle them, the universal spirit animal of the shamans of risk and liability. We're supposed to have goats out there already anyways. I used to live in a different district, and I voted for a MMWD Board member because there was something about their campaign literature that led me to believe that they embraced goats. Maybe it was the fact that their campaign mailer prominently featured a photo of them *literally embracing a goat*. I voted the straight goat ticket, that Board member was elected, but still I've yet to see one goat on our watershed lands. B10-6

A couple more specific suggestions: For one, let's pioneer and trademark the practice of "microburns." We've stated that we are "reintroducing a mosaic of fire" across the landscape, but we actually aren't. Burning piles gives you all the negative repercussions of fire in terms of smoke and air pollution and repercussions for employee health and virtually none of the ecologically beneficial ones, and is extremely labor- and fossil fuels intensive. In fact, some smart person ought to take a hard look at how worthwhile that practice is. It requires revisiting the site many times, and touching and fluffing the material many times, and most of the time you have to put every pile *out cold*, usually accomplished by driving almost 3,000 gallons of treated drinking water to the site, lately up on top of Bolinas Ridge, in at least two different large diesel trucks, with a flotilla of full-size pickups carrying the ignition fuels and the tools and the personnel, etc., and the smoke it generates is *sickening*. I mean, it's sickening to think all that dirty smoke is going *somewhere*, but it is literally sickening for the staff compelled to stand and work in it. Sometimes the air gets trapped in the drainage you're burning in and there's no getting away from it. And often you can't get the engine but so close, so you have to stretch hoses off into the distance until we've sworn we got hose in different area codes, and to keep those hoses charged you have to run the diesel engines at high RPM, sometimes all day. And of course the piles have to season so we have to send someone out to survey them to make sure no nesting birds have moved in, etc., and once as we were taking a pile apart on one of our dam faces to toss it onto another pile we'd managed to get lit under wet, adverse conditions, a Red Legged Frog came hopping out! How many critters, special status or not, do we incinerate prosecuting that ecologically out of sync practice? Let's work with regulators to create a category of burning that doesn't require dozens or hundreds of personnel and dozens and dozens of trucks and apparatus to support it, as the usual prescribed broadcast burn usually does, and conduct small "microburns" frequently that our own staff and apparatus can handle. B10-7

Actually it's probably time for someone to look at our whole fire program and ask: What's appropriate? What are our responsibilities? What do we want our fire program to be? A survey of regional agencies to see what they do should probably be included in such an assessment. What started more than 100 years ago perhaps with a ranger on horseback with a shovel has crept in scope until now we have fire engines and water tenders and even a bulldozer equipped for firefighting and most permanent and even some seasonal staff are trained wildland firefighters. Some of us feel that in spirit the fire program was created so that we could use fire to manage our own natural resources and perhaps respond to incidents on our own lands or maybe, at the way outside, adjacent incidents that *threaten* our lands. Now we sometimes respond to incidents that are nowhere near our lands, and we've even been sent to "cover" other fire stations, whatever that means, of course leaving us without the equipment or personnel to quickly respond to incidents on our own lands. It seems the scope has crept considerably. What's appropriate? Some smart person or persons need to take a clear eyed look at these questions *before* the fact, and communicate their conclusions unequivocally.

Let's turn the fast growing hardiness of Douglas fir trees to our advantage, where appropriate, and let canopy succession occur adjacent to our fire roads. The benefits of canopy cover for reducing erosion are well documented, but also, in those areas where Douglas fir succession adjacent to roads is already a fait accompli, you'll notice that once the high limbing is accomplished – easily taken care of by a single proud industrial athlete with a pole saw -- there is *little to no roadside brushing needed*. The fast growing firs protect the road surface, and obviate almost totally the need for brushing maintenance. Puritanical types may protest that in those zones we want to favor for chaparral, but "favoring" in this instance means that said chaparral will be mechanically obliterated every three to five years by a fourteen ton excavator, and if you're not getting out into the chaparral stands to abate the invading firs – which we're not, because it isn't operationally practicable – then it isn't meaningful or worthwhile to do so adjacent to the roads. Better to leave lower maintenance roads behind us and leave the watershed better than we found it in that respect. Head up the Lagunitas Rock Spring Fire Road all the way to Laurel Dell Fire Road to see varying stages of this succession, or take a trip "to windward" on the Oat Hill Fire Road from the Pine Mountain Fire Road all the way to the end past Old Vee to see what I mean. B10-7

Also, where exclosures are used to foster oak recruitment in particular, let's use the Bough Down-style ephemeral native tree bough box crib exclosures instead of the ones that feature plastic or wire, pressure treated stakes, rubber, etc. At some point someone bought approximately 854,000 blue plastic cylinder exclosures, which have been deployed piecemeal since before I started with MMWD. But they come with woefully inadequate little spindly bamboo stakes about the thickness of a pencil, and they fall over and recruit downhill at a pretty steady rate. There are some at Laurel Dell hanging directly over the creek in one of our SOD plots, and some at our Boneyard near the Bon Tempe Channel, among other places, also right next to the creek, and doubtless some have arrived into the Pacific Gyre by now. The Bough Down style exclosures provoked a strong reaction – one can almost hear the reactionary reasoning: "An exclosure should look like an exclosure" – but the beauty of them is that they use abundant nearby native materials, nothing manmade, and that they are ephemeral. As they moulder away, the plant recruits. Two examples survived, one near the top of the Taylor Trail, and one next to the Pine Point Trail along the Bon Tempe Channel. The latter excloses a volunteer valley oak, scion of some of the best above the dam valley oaks remaining, which are much older than Bon Tempe Lake and indeed visible in MMWD's Acadia publishing book. Such saplings do not survive where deer are abundant, unless they are exclosed. That exclosure was constructed of limbs from nearby fir trees, opening up shoreline sightlines and access, eliminating ladder fuels, etc., etc., a win on many levels. Plastic exclosures are obnoxious in the landscape and in the waterways, and wire ones concentrate EMF's in a way that has turned my forest bathing practice into a living hell. Namaste.

The dearth of granary snag habitat around Sky Oaks causes the acorn woodpeckers to use the wood-sided building and nearby telephone poles for their granary snags (standing dead and sometimes living wood, usually trees, that they riddle with holes over generations in which to cache acorns) and the exposure to paint and chemicals causes them to behave like the teenagers in the vaping commercials. All day long their mocking laughter rains down on the inmates. We have the technology and we certainly have the stock to fashion granary snag features that would be as much landscape art installments as habitat for those wonderful, comical birds, placed so as to focus their activity for the delight of visitors and staff alike, so let's do so. Somewhere around Sky Oaks, for example, perhaps out in the meadow, we ought to have a to scale and properly-oriented Snaghenge, just because.

Issue low-cost permits, as the Forest Service does, so that our own staff, our rate-payers, our volunteers, etc., can cut and take firewood from the watershed. We need all the help we can get when it comes to thinning, and what we do now is to either leave the larger diameter downed wood to moulder away on the mountain side or burn it during the wet season in our Boneyard pile, both of which have regrettable consequences for the quality of the air and the climate. Better to let our stakeholders more cleanly burn seasoned wood to heat their homes, at least until we get biomass digesters that can convert the heavy fuels problem into power. We should make our own charcoal, too, another One Tam branding opportunity. B10-7

Finally, one or more agricultural plots ought to be dedicated and set aside, as SFPUC has done in Sunol, for educational purposes and/or for staff. There's no good reason we shouldn't be a foodshed too, as well as a watershed and cultureshed, etc. Ultimately our static stewards should be able to grow their own, too, if they want. Fruits and veggies *and* Tammy Whammy.

That's all I got for now. To sum up:

Compile and publish a commute map, and update it annually, so we can begin to think about how to shrink it. Informationally submit it to ABAG.

Designate pilot plots for an ultimately watershed-wide network of static stewards, and staff them. Do your part to design for Global Calming. Also, officially recognize and *observe* Global Calming, the international day of stillness and global fossil fuels fast (the essay that elucidates it is attached at the end) slated for the equinox, in recognition of the fact that all these silly quotidian acts of local stewardship, whether they are effective or not, are all for naught if each of us doesn't face these unpleasant and inconvenient facts and make the difficult but by no means impossible changes that we know we *must* to rein in the runaway freight train of climate change.

Investigate biomass like you stated you intended to five years ago, and meanwhile bring weight and volume data capacity online…..Feed the *MAW*!

More stewardship, less science.

Design and construct a connected network of grazed fuelbreaks that grazers, native or not, could move through in a circuit and maintain, perhaps even untended.

Take every opportunity you can to get the fossil fuels out of your stewardship.

And finally, if anyone can name me one single way in which we manage our natural resources in a sustainable manner……I'm all ears!

B10-7

Sincerely,

Lito Brindle

17

Counting down to Global Calming

It's not humans that are warming the planet.

It's human *activity*.

What if all of us could just be still, even for just one day?

Might not be a terrible idea for us Westerners to demonstrate to the rest of the world – many of whom still follow the religious practice of fasting, and to whom we must seem the prodigal children of evolution – that not only do we care about this unprecedented existential threat but that we're capable of exercising a little restraint to address it.

On the vernal equinox of 2019, as the sun passed over the equator on its way back north and the pleasing astronomical symmetry highlighted the confusion we have wrought on the ground, I tried to do just that. Instead of rising long before dawn to drive to work, I rose with the sun. I tried to flip no switch, strain no grid, burn no fossil fuels. I went nowhere my feet couldn't take me. In my little backwater town near the teeming heart of a beclustered California megalopolis, I emphatically sidestepped out of the human gyre and watched it furiously churn. I considered how the quality of the water, and the air, and the climate, die a death by a thousand cuts each day – or rather, 7 or 8 *billion* cuts, since it's safe to say that every human occupying the planet takes at least one action that is deleterious to the quality of the water and the air and the climate, every day – and I simply tried, at least for one day, to make no such cuts of my own.

The day was arbitrarily chosen. The equinox seemed like a good time to ask people around the world to abstain from energy. But there are other fitting, significant days as well. The international Labor Day would be a good one, since it is economic duress that sets almost all of us in motion. Election day here in the US would be apt as well, in recognition of the fact that the decisions we make on that day inordinately affect the fate of the entire planet and all its little live things. But that's the beauty of Global Calming – anyone can participate, and we can stage one at any time. Drop out of international climate accords, when you're one of the worst offenders? Global Calming. Murder and dismember a journalist in your embassy? Why, Global Calming, of course!

We can leave that up to the globally connected consensus. For now, on the outside chance that the powerful don't commit another outrage with impunity in the meantime, let's set our sights on the autumnal equinox, September 23rd, 2019, when the sun passes back across the equator on its way south, for the next Global Calming. Today there are catastrophic, climate-compounded floods here, and corresponding fires in the southern hemisphere. In half a year the situation will be reversed. A green New Deal is fine, but what's really required is a green War. The Pearl Harbor of climate change comes down again and again, and we fail to recognize it. We continue anxiously along with the equivalent of "peace (we might substitute "prosperity") in our time." By September 23rd, 2019, we will be within sight of the year 2020. "Twenty-twenty" is synonymous with perfect sight, so let's make 2020 the year that the scales are struck from our eyes and we face these unpleasant and inconvenient facts with no illusions and unwavering vision.

I once heard a woman describe having been in London after 9/11 when that entire country observed a moment of silence in solidarity with the United States, and she said in a busy square at the teeming heart of the city all that could be heard was the flapping of the pigeons' wings. That's the image to shoot for. The silence will be deafening. The universities and schools, in particular, should have the silence of a graveyard. *That* will get their attention.

More and more people are more and more anxious about the climate situation, but we really don't know what to do about it. Here's something you can do: Participate in Global Calming, an international day of stillness, a global fossil fuels fast. That's what I will be doing on September 23rd, the autumnal equinox of 2019 (if not sooner?). Whether you will be joining us or not, let me be the first to offer the benediction: A peaceful Global Calming to you.

2.4.10 Letter B10: Lito Brindle

Response to Comment B10-1

The comment is noted.

Response to Comment B10-2

As detailed in Section 3.11 Transportation, the Office of Planning and Research identifies the screening threshold of 110 vehicle trips per day (OPR, 2017). The analysis under Impact Transportation-1 has been revised to more accurately reflect the average daily vehicle trips associated with implementation of the BFFIP. Refer to Chapter 3 for these changes. The analysis quantifies the maximum and daily average vehicle trips under the BFFIP. Vehicle trips could not feasibly be innumerable due to level of need and ability to provide services for contractors. Contractors historically have commuted together and, when conducting treatments over a longer period, such as over a week, have camped in Marin County for the duration (Klein, 2017). No significant impacts would occur, and mitigation measures are not proposed.

Response to Comment B10-3

The comment is noted. The management actions proposed as part of the BFFIP are intended to achieve the goals of wildfire risk reduction and increasing biodiversity. The BFFIP was prepared using knowledge collected from decades of vegetation management on District lands and the ever-evolving literature on land management treatments.

Refer to **Master Response 6: Limited Use of Herbicides Alternative** for a discussion of the herbicide alternative and why it was not identified as the environmentally superior alternative.

Response to Comment B10-4

The comment and suggestions are noted. Broadcast burning is proposed under MA-23 through MA-27, as shown in Table 2.9-1. Detailed performance criteria for broadcast burning are identified for MA-23 and MA-24. The impacts of broadcast burning are analyzed as applicable under each resource topic in the EIR. Broadcast burning is becoming an important tool for land managers to address fuel loading and habitat enhancement. The emissions and carbon release from broadcast burning areas of a natural landscape under controlled conditions would be considerably less than the emissions if the area were subject to a wildfire. The benefits of broadcast burning may outweigh the cost of temporary significant emissions during the burn.

Response to Comment B10-5

The comment and suggestions are noted. Refer to **Master Response 5: Grazing** for more information about grazing as a tool for vegetation management under the BFFIP.

Fuelbreaks are proposed as part of the BFFIP. Refer to **Master Response 1: Definition and Location of New and Expanded Fuelbreaks** for information about the proposed fuelbreaks.

Response to Comment B10-6

The comment and suggestions are noted.

Broadcast burning as well as pile burning is proposed under the BFFIP. Broadcast burning would be implemented under MA-23 through MA-27, as shown in Table 2.9-1. Both types of prescribed burning techniques are necessary and achieve different outcomes as part of the BFFIP.

Prescribed burning is analyzed as applicable under each resource topic in the EIR. The significant and unavoidable impacts on air quality and GHG emissions are analyzed in detail and mitigated to the greatest extent feasible, in Section 3.2 Air Quality and Section 3.6 Greenhouse Gases. Impacts on regional air quality and sensitive receptors are described in detail. Broadcast burning contributes most significantly to emissions. Pile burning, vehicles, and equipment emissions are comparatively much lower.

Direct and indirect impacts from prescribed burning on biological resources, including California red-legged frog, are analyzed in Section 3.3 Biological Resources. The analysis details that prescribed burns could kill individual frogs. Mitigation measures require a training program and surveys for California red-legged frog prior to any work involving mechanical equipment or prescribed burning within 0.25 mile of Kent Lake, Lagunitas Creek downstream of Kent Lake, or around Soulajule Reservoir and avoidance of area, if found.

Section 3.12 Energy addresses impacts related to energy use, including via combustion of petroleum products, including gas, diesel, and motor oil. The use of fuel to implement the BFFIP is considered beneficial and necessary and not wasteful given the outcome of the work.

Response to Comment B10-7

The comment and suggestions are noted. Fuelbreaks are proposed as part of the BFFIP. Refer to **Master Response 1: Definition and Location of New and Expanded Fuelbreaks** for information about the proposed fuelbreaks.

Comment Letter B11

From: Larry Bragman <bragmanlaw@gmail.com>
Sent: Wednesday, June 19, 2019 4:39 PM
To: Biodiversity, Fire and Fuels Integrated Plan EIR
Subject: Comments to Draft EIR
Attachments: MMWD BFFIP COMMENTS LB.pdf; MMWD BFFIP Silver et al. 2010 Range Ecology and Management.pdf; MMWD Glyphosate IARC monograph.pdf; MMWD Glyphosate Amphibian Study.pdf; MMWD Glyphosate_Final_Report_Hwang_2011.pdf; MMWD Glyphosate Soil Experiment.pdf

Shaun:

I have attached my comments to the Draft EIR along with the studies that I mentioned. Thank you for your time and consideration.

Larry

--

Law Office of Lawrence Bragman
912 Lootens Place, Second Floor
San Rafael, CA 94901
(415) 459-6060
(415) 459-6067 (Fax)

Comments to the BFFIP EIR

1. The EIR fails to Properly analyze the Use of Grazing for Fire Fuel Reduction

MA-20 to MA-27 describe a number of vegetation management actions. Those actions only mention the use of grazing within MA-27 as an "experimental and trial" basis to determine whether it is suitable for control of invasive species.

In limiting its analysis of grazing for purposes of control of invasive species, ***the study completely fails to consider its value as an alternative for fire safety (fuel reduction) and habitat improvement including by not limited to soil health and carbon sequestration.*** The benefits of grazing for soil health and carbon sequestration were empirically demonstrated by the Marin Carbon Project. See attached: Soil Carbon Pools in California's Annual Grassland Ecosystems Rangeland Ecol Manage 63:128–136 | January 2010 | DOI: 10.2111/REM-D-09-00106.1

Further, the EIR the study doesn't compare the detrimental impact to the environment associated with the CO2 outputs of mechanical versus livestock grazing. The EIR states that the plan's mechanical methods would result in a sizeable (300%) increase in vehicle and equipment usage. This is a significant impact which could be almost entirely avoided with planned grazing.

The study also glosses over the fact that mechanical management is limited to slopes of less than 30% in spite of the fact that there are significant steeply sloped areas within the watershed that are in desperate need of vegetation management. ***Properly herded goats can handle these steeply sloped areas without significant risk or increased costs.***

The EIR also fails to review or consider previous and ongoing successful grazing projects that have already demonstrated their practical and environmental value right here in Marin. For the last two years, the Sleepy Hollow Fire District, Marin County Open Space and several private landowners have conducted cooperative grazing in their adjacent lands. The results were impressive the first year and have improved in year two "Goat grazing can be a cost effective solution to reduce fuels and wildfire hazard in many parts of Marin," according to Rich Shortall, President of both SHFPD and FIRESafe MARIN. https://www.shfpd.org/programs/goat-grazing

The study's bias against goat and livestock grazing is also demonstrated in its consideration of broadcast burning, MA-23 and MA-24. Prescribed and broadcast burning is listed as one of the principle management actions to management vegetation. While anticipated air emissions related to vegetation burning are stated to exceed regulatory thresholds, ***there is no cost, air quality or carbon output comparison of this hazardous method as compared to the alternative use of grazing***. Hence, the study further demonstrates an absence of consideration of grazing as an alternative to strategic fire/burning management practices. B11-1

2. The EIR also underestimates the impacts of the use of herbicides: B11-2

The EIR states, "While the proposed limited herbicide under this alternative has many benefits, it introduces new potential effects that would not occur under the proposed plan. These effects include exposure risks to animals, to humans including applicators and to water quality. *None of these effects would be significant give the limited use of herbicides and the numerous application restrictions....*" This analysis ignores several studies which call into question the safe exposure level for the proposed alternative use of glyphosate.

First, the International Agency for the Research on Cancer concluded that glyphosate was a probable carcinogen for humans and that there was sufficient evidence to conclude that it is carcinogenic as to animals. See attached study IARC monograph at pages 78-79.

Additional studies have demonstrated that glyphosate is toxic as to aquatic species including amphibians. See attached New Effects of Roundup on amphibians: Predators reduce herbicide mortality; herbicides induce antipredatory morphology Releyea, University of Pittsburgh, 2012. B11-2

The EIR analysis of the herbicide "alternative" fails to mention or include MMWD's own 2011 study by Dr. Hwang which demonstrated that ***glyphosate is persistent in the environment***. See attached: Environmental decay of glyphosate in broom-infested Mt. Tamalpais soils and its transport through stormwater runoff and soil column infiltration

Finally, the EIR again demonstrates its glaring failure to analyze the impacts on any of the proposed management actions on soil health and carbon sequestration. Glyphosate is a powerful biocide which has significant impacts on soil health and vitality. Again, this is not even mentioned in the EIR's consideration of herbicides as alternative. See the attached 2014 study, Glyphosate herbicide affects belowground interactions between earth worms and symbiotic mycorrhizal fungi in a model ecosystem.

2.4.11 Letter B11: Larry Bragman

Response to Comment B11-1

Refer to **Master Response 5: Grazing** for more information about grazing as a tool for vegetation management under the BFFIP. Grazing alone cannot replace equipment use or broadcast burning. Many management actions including MA-23 and MA-24, such as Douglas-fir thinning and SOD treatments, would not be achievable with grazing. Broadcast burning is a tool used to address fuel loading and habitat enhancement. As stated under **Master Response 5: Grazing,** under MA-27, the District could perform a study of grazing to understand its efficacy, the resources needed, and environmental impacts and compare these parameters with other methods such as mechanical methods or prescribed fire. The adaptive management approach of the plan could allow for greater use of grazing should data show that grazing is a better tool with fewer impacts.

The commenter cites a long article entitled "Soil Carbon Pools in California's Annual Grassland Ecosystems." The article and its contents are noted. The BFFIP is designed to be a vegetation management program and is not a soil health or carbon sequestration effort. That said, the benefits to soil health from grazing are useful to mention and, therefore, have been added to MA-27 in Chapter 6 of the BFFIP, as follows:

> It should be noted that grazing can also have benefits related to carbon health and increasing carbon sequestration. The expanded use of grazing could be incorporated into the plan to enhance these benefits.

The impacts related to equipment and vehicle use are analyzed throughout the EIR, as applicable. All impacts associated with use are mitigable to less than significant levels. No significant and unavoidable impacts related to equipment and vehicle use are identified. As noted by the commenter, MM Geology-1 restricts heavy equipment use to slopes of 30 percent or less to prevent erosion and destabilization. Hand-held mechanical equipment or manual methods can still be employed in these areas, as could goats under MA-27.

The significant and unavoidable impacts on air quality and GHG emissions are analyzed in detail and mitigated to the greatest extent feasible in Section 3.2 Air Quality and Section 3.6 Greenhouse Gases. Broadcast burning contributes most significantly to emissions. Vehicles and equipment emissions are comparatively much lower and in isolation would not result in a significant and unavoidable impact. Grazing does not offer the same ecological benefits of prescribed burning and cannot replace it as a management tool.

Section 3.12 Energy addresses impacts related to energy use, including via combustion of petroleum products including gas, diesel, and motor oil. The use of fuel to implement the BFFIP is considered beneficial and necessary and not wasteful given the outcome of the work.

The successful grazing projects identified by the commenter are noted. The BFFIP allows for grazing under MA-27 and grazing is fully analyzed throughout the Draft EIR in equal detail as mechanical and manual methods and prescribed burning. Should experimental trials show grazing to be successful, use of grazing can be expanded under adaptive management.

Response to Comment B11-2

Refer to **Master Response 6: Limited Use of Herbicides Alternative** for a discussion of the herbicide alternative, including the varying conclusions about the health risks associated with glyphosate use, based on various scientific studies. While the Draft EIR does not specifically address the scientific article referenced by commenter, several studies identifying glyphosate as likely and not likely carcinogenic are provided in Master Response 6: Limited Use of Herbicides Alternative, demonstrating the conflicting evidence available. The method and amount of application can also influence the level of effects. For example, the limited use would not result in substantial areas of soil exposure to glyphosate that could experience the effects identified in the studies referenced.

The EIR concludes that the herbicide alternative would have additional risks to humans and the environment and is not the environmentally superior alternative.

From: Larry Bragman <bragmanlaw@gmail.com>
Sent: Wednesday, June 19, 2019 5:00 PM
To: Biodiversity, Fire and Fuels Integrated Plan EIR
Subject: Re: Comments to Draft EIR
Attachments: MMWD BFFIP Insect Control.pdf; MMWD BFFIP Insect Control 2.pdf; MMWD Campaign Broom Bug Ford.pdf

Comment Letter B12

One additional alternative that should be considered is experimental study of insect predators for the control of invasives. I have attached some studies which discuss this method. B12-1

Thanks,

Larry Bragman

On Wed, Jun 19, 2019 at 4:39 PM Larry Bragman <bragmanlaw@gmail.com> wrote:

Shaun:

I have attached my comments to the Draft EIR along with the studies that I mentioned. Thank you for your time and consideration.

Larry

--

Law Office of Lawrence Bragman
912 Lootens Place, Second Floor
San Rafael, CA 94901
(415) 459-6060
(415) 459-6067 (Fax)

--

Law Office of Lawrence Bragman

1

912 Lootens Place, Second Floor
San Rafael, CA 94901
(415) 459-6060
(415) 459-6067 (Fax)

2.4.12 Letter B12: Larry Bragman

Response to Comment B12-1

The suggestion is noted. MA-27 would involve implementation of weed control trials. Weed control trials would be used to study the efficacy of available techniques to determine whether they would be appropriate for use on a larger scale. The District could consider whether to support studies of insect predators for control of invasive plant species as part of MA-27 (noting that the study of insect predators and biocontrols is the purview of the U.S. Department of Agriculture Animal and Plant Health Inspection Service and the California Department of Food and Agriculture). Adoption of the BFFIP as proposed does not preclude experimental study of insect predators; however, additional, tiered environmental review may be required as the Draft EIR does not directly address the impacts of biological control agents.

Comment Letter B13

From: Aaron W. Gilliam <agcypress@gmail.com>
Sent: Wednesday, June 19, 2019 6:47 PM
To: Biodiversity, Fire and Fuels Integrated Plan EIR
Subject: Draft EIR public comment from a livestock operator

Hello Shaun,
I currently work 7 days a week without a desk or computer access, most of my time being spent out in the field with no internet and often little cell reception, tending to my flocks of sheep on their grazing contracts. If you are able to consider my slightly tardy public comment I would greatly appreciate it. B13-1

I would like the EIR review team to consider that the Grazing Feasibility Study published in June o 2018 may have excluded some creative options for making grazing more feasible as a grassland management tool.

I am glad to see that they considered the combined approach of utilizing both mechanical removal and grazing as a preferred technique, as I believe this is certainly the case for most of the weedy species within the MMWD. It was also good to see that monitoring and adaptation of grazing plans was emphasized as a necessity for success.

As one of the contract grazers that was contacted, I can confidently say that the price scale that the Feasibility study focused on was skewed to the high end. They were interested in knowing the price of grazing in the most expensive seasons when forage quality and water availability is low and demand for services is high. Grazing management for desired grassland species is often best accomplished in the green season (our slow season), when water is still available on the landscape and the cost of contract grazing can be considerably lower. B13-2

There are two other ideas that I think should be brought up as creative ways to bring the cost of management down. One would be to have extra grazing staff operating as the labor force providing manual removal of target weedy species. They already have to be on site watching over the sheep and tending camp. They could be more cost effectively deployed to do this work. The second idea involves agro-tourism where the income of the grazing operator could be augmented by allowing them to host paid, educational, public-participation events. The public would participate in manual removal of target weeds and help the shepherds with herding the flocks while receiving custom eating and camping opportunities otherwise only permitted to the employees of the grazing company.

Finally, please consider that having a resident flock on MMWD land that would not be shipped out and back multiple times a year, but would stay on the land and get herded to the various target sites multiple times would be most cost effective. This would require more planning but would also achieve better results in overall grassland health that can not be achieved if animals are only brought on during the dry season or target weed season which is also often during the dry months.
I would be happy to provide more thorough input in person or in writing. Thank you for considering these opinions. If you are unable to do so, I understand, but thank you for at least reading.

Thank you,
Aaron Gilliam

Owner
Sweetgrass Grazing
(805)450-5408

1

2.4.13 Letter B13: Aaron W. Gilliam

Response to Comment B13-1

The comment is being considered and is addressed.

Response to Comment B13-2

The comment and suggestions are noted. Refer to **Master Response 5: Grazing** and Response to Comment B11-1 for more information about grazing as a tool for vegetation management under the BFFIP. The BFFIP allows for grazing under MA-27, and grazing is fully analyzed throughout the Draft EIR in equal detail as mechanical and manual methods and prescribed burning. Should experimental trials show grazing to be successful, use of grazing can be expanded under adaptive management. The costs of grazing are beyond the scope of the EIR.

2.5 RESPONSES TO PUBLIC MEETING COMMENTS

PUBLIC MEETING

Comments C1, C2, C3, and C4

PUBLIC MEETING

Date: April 10, 2019 – 7:00 pm

Location: Marin Municipal Water District Main Office

Project: Biodiversity, Fire, and Fuels Integrated Plan Draft Program Environmental Impact Report (DEIR)

Subject: Public Comments on the Draft Program EIR

Summary

During the public meeting on April 10, 2019, Marin Municipal Water District Vegetation Ecologist Andrea Williams and Natural Resources Manager Shaun Horne presented to the public a summary of the Biodiversity, Fire and Fuels Integrated Plan and the DEIR. Four residents provided comments on the DEIR. The following section is the transcript of the relevant portion of the meeting recording from court reporter when community members provided public comments.

Transcript

Nona Dennis: My name is Nona Dennis and I'm representing Marin Conservation League. We haven't had time to really do a through examination of 724 pages yet. And, but we have some preliminary interest. We've been tracking this process for more than ten years. And we've been waiting rather impatiently for the EIR to come out. We'd really like the plan itself to go forward. I realize that you're not really taking comments on the plan tonight, although sometimes we confuse the plan with the EIR. On the plan itself, I will continue to say that there is one deficiency, one area which is not addressed by the plan. And that is the what I call the non water producing side of MMWD, which is the south and south-facing slope in which senescent chaparral, which is building up fuel at a furious rate, including Douglas, Fir, is not addressed in the plan. C1-1

Moving to the EIR, I really want to commend the District for what on first glance appears to be a very comprehensive EIR. And I've seen a lot of EIRs in my career. But, I particularly I went back to MCL's requests at the scoping period to see whether you had addressed our requests. And they were fairly rigorous. But the main one really was you include in the EIR a limited herbicide alternative. And I want to commend you for doing that. We want to commend you. We did ask also for quantification. We wanted to know really in a comparative way what difference it would make to incorporate limited herbicide use in a targeted way, what difference that would make in the, let's say, acres covered per time unit, per human unit, labor unit and so forth. And we don't see that yet. We may ask in more specific detail that we would like to have that because there is the risk of continuing business as usually now without herbicide which could lead to increased, actually increased risk of fuel buildup and so forth. But as I went through the various topics that are covered and discussed under that alternative, it appears that the decision to not use herbicides is really primarily a social and political one. The environmental, the EIR actually finds that there are no significant impacts from using herbicides in a limited way under any category. Doesn't change the finding for air C1-2

Page | 1

PULIC MEETING

quality or for greenhouse gas emissions, which will continue to be significant and unavoidable. But it does really, it does say that there will be some, in spite of an impact let's say on water quality being less than significant. It does say that there will be an increased amount of impact, but it still falls under the less than significant impact. So we would commend you for having an objective review. You did what we wanted you to do up to the point of not quantifying the impact. But we feel that you have fairly and openly, objectively, included that alternative. And we find that is would be nice to pursue it. But we appreciate that you've included it in the EIR. Thanks. C1-2

Ginger Souders-Mason: Ginger Souders-Mason, Director of Pesticide Free Zone. And I want to thank you very much for eliminating the herbicide alternatives because we know now that the feeding with goats and some sheep, is very, very effective and also reduces the amount of greenhouse gas production through the use -- non use of herbicides which are petrochemical derivatives. C2-1 I have a question and I must confess that I have not picked up the tome to look at it yet, which I will do. And I realize that there aren't many places where PG&E lines cross MMWD property. But I was hoping that in the report somewhere you are discussing that interaction between the equipment of PG&E and which has failed in many places, and the work that you do in terms of maintaining your grasslands and areas in a safe manner. Nothing else, other than to thank you very much for all the years of work. And I'm sorry the people who produced the tome are no longer with you. But, I'm glad you're here. C2-2

Larry Minikes: Larry Minikes, San Raphael. One thing about goats is that they will avoid broom and broom is our probably our worst invasive up there. So the use of goats is not going to change that dynamic, unfortunately. So we do have to look at other alternatives for the reasons that Nona Dennis pointed out. C3-1 My concern, or the concern that the public should have, is that it's the idea of reducing fuels, of protecting improving the wildland- urban interface is very, very important. C3-2 But we have to make sure that we're going to properly fund it. And that is probably key to any plan. You can have the best plan in the world, but if you can't go through with it, what good is it? So that's the thing that I think the public really has to get behind is to see that we find whatever mechanism we need to fund this, whether it is grants, whether it's however. And fund the plan properly. Thank you. C3-3

Eva Buxton: I just have a short comment. Eva Buxton, CNPS, Native Plant Society. Yes, I've seen in many documents over the years where we talk about using herbicides. The word "limited" is always used. And so I've seen written out the screen, "limited use of herbicides." Well, what does "limited" mean? That has to be defined. Is it limited to one year? Is it limited to one certain plant? Is it limited in amount that you can spray over an area? "Limited" has no meaning unless you talk about what "limited" means or where it can be used or where herbicides can be used. And that's just my comment. I hope that would be addressed. Thank you. C4-1

2.5.1 Commenter C1: Nona Dennis, Marin Conservation League

Response to Comment C1-1

The commenter appears to be referring to the area on District lands within the Redwood Creek Watershed that does not drain into the reservoirs. In Chapter 2 Project Description of the Draft EIR, Figure 2.6-4 identifies the fuelbreaks within this area as mostly Transitional, and Figure 2.6-9 identifies much of this area as an Ecosystem Preservation Zone intermixed with Ecosystem Restoration Zone. The areas identified as Ecosystem Preservation Zone are largely intact ecosystems with the long-term strategy to maintain existing conditions without increasing effort. Table 2.7-1 in Chapter 2 Project Description of the Draft EIR, identifies the management actions that would involve treatment within Transitional Fuelbreaks and the Ecosystem Restoration Zone. Maintenance and creation of fuelbreaks would occur in this area under MA-20 and MA-21. The Douglas-fir thinning and broadcast burning proposed under MA-24 would occur within the Ecosystem Restoration Zone.

Response to Comment C1-2

Refer to **Master Response 6: Limited Use of Herbicides Alternative** for a discussion of the herbicide alternative and why it is not superior to the BFFIP as proposed. According to the CEQA Guidelines, the "EIR shall include sufficient information about each alternative to allow meaningful evaluation, analysis, and comparison with the proposed project." The description and analysis of an alternative does not need to be as robust as the proposed project (Section 15126.6; County of Inyo v. City of Los Angeles (1981)124 Cal.App.3d 1). Chapter 4 Alternatives to the Proposed Plan of the Draft EIR evaluates the alternatives with respect to consistency with plan objectives, feasibility, and environmental effectiveness. The effectiveness of each alternative retained for analysis, including the Limited Use of Herbicides Alternative, to meet the plan objectives is summarized in Table 4.5-1 of the Draft EIR and within the analysis for each alternative.

2.5.2 Commenter C2: Ginger Souders-Mason, Pesticide Free Zone

Response to Comment C2-1

Refer to **Master Response 6: Limited Use of Herbicides Alternative** for a discussion of the herbicide alternative and why it was not identified as the environmentally superior alternative.

Response to Comment C2-2

The comment is noted. PG&E performs maintenance of vegetation for safety within the power line rights-of-way. Ongoing maintenance activities conducted by PG&E are a part of the baseline conditions. Existing coordination between the District and PG&E would continue to occur following implementation of the BFFIP.

MA-24, proposed as part of the BFFIP, would involve vegetation management activities intended to improve grassland habitat. Broadcast burning would be conducted in grasslands, which would minimize fuel loads and spread of invasive species. Douglas-fir thinning and targeted invasive plant species removal would also occur within grasslands.

2.5.3 Commenter C3: Larry Minikes

Response to Comment C3-1

Refer to **Master Response 5: Grazing** for more information about grazing as a tool for vegetation management under the BFFIP. Several management actions proposed under the BFFIP, including MA-22, MA-24, and MA-27, would involve management and removal of invasive plant species using varying tools and techniques. Grazing would constitute only a small portion of the proposed invasive species management. Other tools and techniques proposed including hand pulling, use of equipment, and broadcast burning.

Response to Comment C3-2

The WUI around District lands is identified and discussed in Section 3.7 Hazardous Materials and Fire Hazards of the Draft EIR. One of the goals of the BFFIP is to minimize risk of wildfires, particularly due to the large number of structures and residents within the WUI. Fuelbreak maintenance and construction and fuel load control in WAFRZ, proposed under MA-20, MA-21, and 23, would minimize wildfire risks, including for those in the WUI. Refer to **Master Response 1: Definition and Location of New and Expanded Fuelbreaks** for description of fuelbreaks and WAFRZ.

Response to Comment C3-3

The comment is noted. The cost of the BFFIP is not relevant to the analysis of environmental impacts under CEQA. See Response to Comment A3-11.

2.5.4 Commenter C4: Eva Buxton, Marin Chapter of the California Native Plant Society

Response to Comment C4-1

Refer to **Master Response 6: Limited Use of Herbicides Alternative** for a discussion of the herbicide alternative and why it was not identified as the environmentally superior alternative. The description of the Limited Use of Herbicides Alternatives in Chapter 4 Alternatives to the Proposed Plan in the Draft EIR includes many limitations and restrictions. Refer to the various bulleted and numbered lists in Section 4.5.4 of the Draft EIR.

2.6 REFERENCES

Alling, C. E. (2011). *Deferring Mitigation Measure Details What is and is not Allowed by CEQA?* Ascent.

ATSDR. (2019, April). Toxicological Profile for Glyphosate Draft for Public Comment.

Cobb, R. C., Hartsough, P., Ross, N., Klein, J., LaFever, D. H., Frankel, S. J., & Rizzo, D. M. (2017). Resiliency or Restoration: Management of Sudden Oak Death Beofe and After Outbreak. *Forest Phytophthoras*, 1-14.

Davison, J. C., Smith, E., & Wilson, L. M. (2007). Livestock Grazing Guidelines for Controlling Noxious Weeds in the Western United States.

District. (2019). *Tamalpais Lands Collaborative (TLC)*. Retrieved from https://www.marinwater.org/289/Tamalpais-Lands-Collaborative-TLC

Edson, E., S., F., Fish, A., Gardali, T., Klein, J., Kuhm, W., . . . Williams, A. (2016). Measuring the Health of a Mountain: A Report on Mount Tamalpais' Natural Resources.

Ettlinger, E. (2019, July 17). Marin Municipal Water District Aquatic Ecologist. (J. Schweitzer, Interviewer)

Grabow, L. (2012, Octber 29). Personal Communication. (K. Black, Interviewer)

Hamm, K. A., & Diller, L. V. (2009). Forest management effects on abundance of woodrats in northern California. *Northwestern Naturalist*, 90: 97 - 106.

Hansen, E. (2019). *Efficacy of Local Eradication Treatments Against the Sudden Oak Death Epidemic in Oregon Tanoak Forests.* Corvallis: Oregon State University.

Hosking, J. R., Smith, J. M., & Sheppard, A. W. (1996). The biology of Australian weeds. Cytisus scoparius (L.) Link subsp. scoparius. *Plant Protection Quarterly*, 102-108.

IARC. (2017). Some Organophosphate Insecticides and Herbicides Volume 112. *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*. World Health Organization International Agency for Research on Cancer.

Kalies, E. L., & Kent, L. L. (2016). Tamm Review: Are fuel treatments effective at achieving ecological and social objectives? A systematic review . *Forest Ecology and Management*, 84-95.

Klein, J. (2017, April 3). Natural Resources Program Manager, MMWD. (C. Gilleran, Interviewer)

Leonard Charles Associates and Wildand Resource Management. (2008). *Marin Municipal Water District Vegetation Management Plan Update Background Report No. 6 Fire Hazard Management.* Corte Madera: MMWD.

Martinson, E. J., & Omi, P. N. (2013, June). *Fuel Treatments and Fire Severity: A Meta-Analysis.* USDA.

Merriam, K. E., Keeley, J. E., & Beyers, J. L. (2007). The Role of Fuel Breaks in the Invasion of Nonnative Plants. USGS.

National Pesticide Information Center. (n.d.). Triclopyr Fact Sheet. Retrieved from http://npic.orst.edu/factsheets/triclogen.pdf

OEHHA and CalEPA. (2008). *Indicators of Climate Change in California*. Retrieved from https://oehha.ca.gov/media/downloads/climate-change/report/2018caindicatorsreportmay2018.pdf

OPR. (2017, November). Technical Advisory on Evaluating Transportation Impacts in CEQA.

UC Berkeley. (2019). *Does glyphosate (Roundup and other trade names) enhance P. ramorum (sudden oak death) infection?* Retrieved from UC Berkeley Forest Pathology and Mycology Lab: https://nature.berkeley.edu/garbelottowp/?sp_faq=does-glyphosate-roundup-and-other-trade-names-enhance-p-ramorum-sudden-oak-death-infection

USEPA. (2017, December 12). Revised Glyphosate Issue Paper: Evaluation of Carcinogenic Potential. EPA's Office of Pesticide Programs.

Zevit, P. (2019, September 17). *Biodiveristy of BC*. Retrieved from ibis.geog.ucb.ca: https://ibis.geog.ubc.ca/biodiversity/BiodiversityandInvasiveSpecies.html

3 REVISIONS TO TEXT OF DRAFT EIR

3.1 INTRODUCTION

This chapter presents revisions that have been made to the Draft Program EIR text. These revisions provide corrections, additions, or clarifications. The text revisions are organized by resource topics. Underlined text represents language that has been added to the Draft Program EIR; ~~text with strikethrough~~ has been deleted from the Draft Program EIR.

3.2 DRAFT EIR REVISIONS

3.2.1 Executive Summary

All references to MM Biology-9 in Table ES-1 on pages ES-27 through ES-41 are revised as follows:

> MM Biology-9: Protection of Western Pond Turtle Nesting and Overwintering Habitat

Table ES-1 on pages ES-27 through ES-41 is revised as follows:

Impact Statement	Level of Significance before Mitigation	Mitigation Measures	Level of Significance after Mitigation
Biological Resources			
Impact Biology-1: The proposed plan could have a substantial adverse effect, either directly or through habitat modifications, on any species identified as a candidate, sensitive, or special-status species in local or regional plans, policies, or regulations, or by CDFW or USFWS.	Potentially significant	**MM Biology-1: Worker Training** An environmental training program shall be developed and presented by a qualified biologist to all vegetation management workers before they are allowed to perform work under the BFFIP. The training shall describe special-status species and sensitive habitats that could occur within vegetation management areas, protection afforded these species and habitats, and the avoidance and minimization measures required to avoid and/or minimize impacts on these species and habitats, including maintaining avoidance areas, identification of species for avoidance, and protocols to follow, including protocols for minimizing the spread of invasive species and forest diseases. **MM Biology-2: Protection of Special-Status Plants** The following measures shall be implemented to protect special-status plants: c. Prior to conducting any vegetation management activity (mechanical or manual removal), prescribed (broadcast and pile) burning, propane flaming, and animal grazing the area shall be reviewed by the District's botanist against the most current mapping data of special-status plant species and habitats. If the work is to occur in in serpentine habitat, within 500 feet of known special-status plant populations, near wetlands, or within other habitats with potential to support special-status plant populations, botanical surveys shall be conducted by a qualified botanist ahead of the planned work. The surveys shall be specific to the species of plants that could occur, must be conducted during a period when the special-status species that could occur in that habitat can be most readily detected (e.g. blooming period), and shall include the entire footprint of the proposed work. Any species identified during surveys shall be added to the GIS of current mapping data. ~~Areas only need to be surveyed within the previous 5 years.~~ If work is to occur again in the same area within 5 years (e.g., new fuelbreaks or	Less than significant with mitigation

Impact Statement	Level of Significance before Mitigation	Mitigation Measures	Level of Significance after Mitigation
		retreatment areas for forestry actions), a new survey is not required. ~~d. For special-status species of low sensitivity ranking and that are common on District lands and resilient to disturbance (e.g., Mount Saint Helena morning-glory), disturbances shall be minimized to the degree practical but complete avoidance is not necessary, as directed by the MMWD botanist.~~ b. For listed species ~~of moderate or high sensitivity ranking,~~ with known rarity or declining including CRPR Rank 1B, 2, and some rank 4 species that are known rare), as determined and listed below by the MMWD botanical staff ~~(but not limited to this list)~~, the MMWD's botanical staff shall ~~identify the appropriate avoidance measures to be implemented based on the life form~~: i. Flag or otherwise demarcate the individual or population to ensure workers avoid the species for no loss of individuals. ii. Establish a buffer of 100 feet around the individual or population. iii. Require implementation of BMP-1 through BMP-3 for work conducted adjacent to these species to minimize the spread of invasive species. • Brewer's milk vetch (*Astragalus breweri*) • Brewer's calandrinia (*Calandrinia breweri*) • Johnny-nip (*Castilleja ambigua var. ambigua*) • Marin western flax (*Hesperolinon congestum*) • Bristly leptosiphon (*Leptosiphon acicularis*) • Thin-lobed horkelia (*Horkelia tenuiloba*) • Small groundcone (*Kopsiopsis hookeri*) • Gairdner's yampah (*Perideridia gairdneri ssp. gairdneri*) • North coast semaphore grass (*Pleuropogon hooverianus*) • Marin manzanita (*Arctostaphylos virgata*)	

<table>
<tr><th>Impact Statement</th><th>Level of Significance before Mitigation</th><th>Mitigation Measures</th><th>Level of Significance after Mitigation</th></tr>
<tr><td></td><td></td><td>

<u>Santa Cruz microseris (Stebbinsoseris decipiens) *</u>
<u>Coast rockcress (Arabis blepharophylla)</u>
<u>Pink star-tulip (Calochortus uniflorus)</u>
<u>Glory brush (Ceanothus gloriosus var. exaltatus)</u>
<u>Mason's ceanothus (Ceanothus masonii)</u>

<u>* This species is likely extirpated</u>
<table>
<tr><th><s>Species</s></th><th><s>Life Form</s></th></tr>
<tr><td><s>Mount Tamalpais oak (1B.3)</s></td><td><s>Perennial evergreen shrub</s></td></tr>
<tr><td><s>Mount Tamalpais manzanita (1B.3)</s></td><td><s>Perennial evergreen shrub</s></td></tr>
<tr><td><s>Marin manzanita (1B.2)</s></td><td><s>Perennial evergreen shrub</s></td></tr>
<tr><td><s>Glory brush (4.3)</s></td><td><s>Perennial evergreen shrub</s></td></tr>
<tr><td><s>Mason's ceanothus (SR, Rank 1B.2)</s></td><td><s>Perennial evergreen shrub</s></td></tr>
<tr><td><s>Western leatherwood (1B.2)</s></td><td><s>Perennial deciduous shrub</s></td></tr>
<tr><td><s>Napa false indigo (Rank 1B.2)</s></td><td><s>Perennial deciduous shrub</s></td></tr>
<tr><td><s>Serpentinite reed grass (4.3)</s></td><td><s>Perennial herb</s></td></tr>
<tr><td><s>Mount Tamalpais thistle (1B.2)</s></td><td><s>Perennial herb</s></td></tr>
<tr><td><s>California bottle-brush grass (4.3)</s></td><td><s>Perennial herb</s></td></tr>
<tr><td><s>Thin-lobed horkelia (1.B.2)</s></td><td><s>Perennial herb</s></td></tr>
</table>
</td><td></td></tr>
</table>

Impact Statement	Level of Significance before Mitigation	Mitigation Measures	Level of Significance after Mitigation

~~Small groundcone (2B.3)~~	~~Perennial herb~~
~~Marsh zigadenus (Rank 4.2)~~	~~Perennial bulbiferous herb~~
~~Oakland star-tulip (4.2)~~	~~Perennial bulbiferous herb~~
~~Tiburon buckwheat (1B.2)~~	~~Annual herb~~
~~Marin western flax (FT, ST, Rank 1B.1)~~	~~Annual herb~~
~~Tamalpais lessingia (1B.2)~~	~~Annual herb~~
~~Marin County navarretia (Rank 1B.2)~~	~~Annual herb~~
~~Tamalpais jewel-flower (1B.3)~~	~~Annual herb~~
~~Mount Tamalpais bristly jewel-flower (1B.2)~~	~~Annual herb~~

c. For other listed species of CRPR rank 1B or 2 (beyond those identified in part b, above) with the potential to occur on District lands, the following measures shall be implemented

i. Perennials:

1) Mark populations in the field with distinct flagging. Ensure that worker training is complete per MM Biology-1.

2) Avoid populations. If mowing cannot be safely performed up to the perimeter of the individuals, or timed for when they are senescent, then hand methods (i.e., hand pulling or use of non-powered or powered hand tools) shall be employed to prevent damage or removal of listed species.

3) Where tree or shrub species must be trimmed, such as Mount Tamalpais manzanita, follow any protocols or recommendations available, ~~such as~~ including the following the *Status and Management Recommendations for Arctostaphylos virgata (Marin*

Impact Statement	Level of Significance before Mitigation	Mitigation Measures	Level of Significance after Mitigation
		Manzanita) in Point Reyes National Seashore (Parker, 2007) and plant specific pruning tips (Las Pilitas Nursery, 2012) and perform the work by hand. 4) No net loss of an annual special-status species can occur. The population size shall be determined from the most recent survey data of the species. 5) If an individual or population must be removed, one or two options can be employed (subject to CDFW approval) and monitoring conducted to ensure that no net loss of the species occurs. • (1)The individual or population can be dug up and relocated to appropriate habitat outside the work area. (2) A nursery with experience growing special-status plants can be employed to grow seedlings of the species that shall be planted in appropriate habitat outside the work area or in the work area following completion of work. If located outside the work area, appropriate habitat shall be within the same watershed as the impact area, and shall be identified or approved of by MMWD botanical staff. • A monitoring plan shall be developed that details the following components. Conduct annual monitoring of seeded or replanted locations for a minimum of 3 years and up to 5 years, dependent upon the MMWD botanical staff recommendation and monitoring results. If the new population is not matching the pre-removal population data, more seeding or planting shall be conducted until pre-removal population is met ii. Annuals: 1) Flag or otherwise demarcate and ensure workers avoid the species as feasible; or, 2) Time vegetation management activities for when the special-status species occurring in the work area is senescent and/or after the seed has set.	

Impact Statement	Level of Significance before Mitigation	Mitigation Measures	Level of Significance after Mitigation
		3) Monitor populations between vegetation management activities to ensure that population sizes are not decreasing. If populations are decreasing and a correlation can be made to the maintenance activities, measures shall be identified by MMWD botanical staff and taken to improve the population, ~~such as~~ including but not limited to one of the following: avoiding the area in question or altering the management activity frequency. 4) No net loss of an annual special-status species can occur. The population size shall be determined from the most recent survey data of the species. If an individual or population must be removed, one or two options can be employed and monitoring conducted to ensure that no net loss of the species occurs/ • (1) Seeds of the annuals shall be collected from existing on-site populations or from the same watershed (to maintain local genetic stock) and distributed in appropriate habitat outside the work area (within the same watershed) or in the work area following completion of work. (2) A nursery with experience growing special-status plants can be employed to grow seedlings of the species (from seeds collected locally) that shall be planted in appropriate habitat outside the work area or in the work area following completion of work. It should be noted that seeds derived from plants in the same watershed as the impact area may be available from local nurseries, and local nurseries may also be able to propagate seeds from adults grown from collected seeds. In this case, seeds do not need to be collected from a specific impact area site. Appropriate habitat shall be identified or approved of by MMWD botanical staff. • A monitoring plan shall be developed that details the following components. Conduct annual	

Impact Statement	Level of Significance before Mitigation	Mitigation Measures	Level of Significance after Mitigation
		monitoring of seeded or replanted locations for a minimum of 3 years and up to 5 years, dependent upon the MMWD botanical staff recommendation and monitoring results. If the new population is not matching the average population data, more seeding or planting shall be conducted until pre-removal population levels are met. **MM Biology-3: Prevent the Spread of Invasive Species** Precautions shall be taken to minimize the introduction of any invasive weeds or to prevent the spread of existing infestations. Prior to conducting an activity that requires the use of mechanical equipment; the area shall be reviewed by a qualified biologist against the most recent maps of invasive species infestation. The biologist shall direct the work crews as to the need for vehicle cleaning and/or the order in which work should be conducted to minimize the possible spread of invasive species. If work is to commence in an area of known invasive species infestation, the work shall be limited to the area of infestation and no equipment shall move to uninfested areas without being washed first. Alternatively, work shall start in the uninfested areas and progress to the more heavily infested areas last. Areas of broadcast burns shall be monitored annually to ensure that invasive species/weeds are not taking over. Invasive species shall be removed until native vegetation establishes. **MM Biology-4: Prevent the Spread of Forest Diseases from Plan Activities** Forest disease spread shall be evaluated by District biologists when management actions are being performed. ~~on an annual or more frequent basis, as dictated by the progression of the disease and the amount of habitat or vegetation impacted~~. An evaluation shall be triggered when a District biologist observes that a native vegetation type within the BFFIP area has been impacted by the disease. The biologists shall determine if mechanical methods of vegetation removal could result in the	

Impact Statement	Level of Significance before Mitigation	Mitigation Measures	Level of Significance after Mitigation
		spread of the disease in a given project area, prior to implementing the project. This evaluation shall be conducted by looking at the location of the disease, the types of species that are being impacted, and the methods by which the disease is spreading. If the disease is spread by soil contact, then the biologist shall prescribe methodologies for reducing spread from mechanical methods of vegetation management. These methods would likely be similar to those identified in BMP-4 through BMP-7 including, but not be limited to, washing equipment after working in infected areas, and planning work to progress from uninfected areas to infected areas. **MM Biology-5: Roosting Bats** ***Broadcast Burning*** Prior to conducting broadcast burning, a qualified biologist shall review the selected location to determine whether potential roosting bat habitat is present. If adequate roosting trees are present, one of two options may be pursued: (1) A qualified bat biologist shall first conduct a focused assessment of the roosting habitat within 2 days of burning to determine whether bats are present. If bats are present. the bat biologist shall determine whether the broadcast burn poses a threat to the roosting bats based on the location of the bats as compared with the prescribed burn location, wind directions, and type of fuel to be burned. If bats could be within direct line of smoke, a threat would occur If a threat could occur, the broadcast burn must be conducted when ambient temperatures are warmer to allow escape of the bats or the tree(s) avoided. (2) The broadcast burn will be conducted, avoiding the potential roosting trees. ***Tree Removal*** Prior to the removal of trees with a DBH of greater than 10", a qualified biologist shall conduct a focused tree habitat assessment. Trees containing suitable potential bat roost habitat features shall be clearly marked or identified. If day roosts are found to be potentially present, the biologist shall prepare a site-specific roosting bat protection plan to be implemented. Based	

Impact Statement	Level of Significance before Mitigation	Mitigation Measures	Level of Significance after Mitigation
		on site-specific conditions, the plan should incorporate the following guidance as appropriate: **Roost Avoidance** When possible, removal of trees identified as providing suitable roosting habitat should be conducted during seasonal periods of bat activity, including: • Between March 1 and April 15, or after evening temperatures rise above 45 degrees Fahrenheit and/or no more than ½ inch of rainfall within 24 hours occurs; or • Between September 1 and about October 15, or before evening temperatures fall below 45 degrees Fahrenheit and/or more than ½ inch of rainfall within 24 hours occurs. If it is determined that a colonial maternity roost is potentially present, the roost shall be avoided and shall not be removed during the breeding season (April 15 to August 31) unless removal is necessary to address an imminent safety hazard. Operation of mechanical equipment producing high noise levels (e.g., chainsaws, heavy equipment) in proximity to buildings/structures supporting or potentially supporting a colonial bat roost shall be restricted to periods of seasonal bat activity (as defined above), when possible. **Assessment** If work with loud, mechanical equipment must occur near a known or potential roosting structure/building during the maternity or hibernation roosting periods, then a qualified bat biologist shall first conduct a focused assessment of the structure. The site-specific plan shall be implemented to prevent noise-related impacts on roosting bats. **Roost Removal** If a tree potentially containing a colonial maternity roost must be removed, such as in the event of unsafe conditions requiring treatment, during the breeding season, then the following or other measures recommended by the qualified bat biologist may be implemented:	

Impact Statement	Level of Significance before Mitigation	Mitigation Measures	Level of Significance after Mitigation
		• Acoustic emergence surveys or other appropriate methods shall be conducted/implemented to further evaluate if the roost is an active maternity roost. • If it is determined that the roost is not an active maternity roost, then the roost may be removed in accordance with the other requirements of this measure; • If it is found that an active maternity roost of a colonial roosting species is present, the roost shall not be disturbed during the breeding season. Potential colonial hibernation roosts will only be removed during seasonal periods of bat activity (i.e., non-hibernation periods). Potential non-colonial roosts that cannot be avoided shall be removed on warm days in late morning to afternoon when any bats present are likely to be warm and able to fly. Appropriate methods shall be used to minimize the potential of harm to bats during tree removal. Such methods may include using a two-step tree removal process. This method is conducted over two consecutive days, and works by creating noise and vibration by cutting non-habitat branches and limbs from habitat trees using chainsaws only (no excavators or other heavy machinery) on Day 1. The noise and vibration disturbance, together with the visible alteration of the tree, is very effective in causing bats that emerge nightly to feed, to not return to the roost that night. The remainder of the tree is removed on Day 2. **MM Biology-6: Protection of Badgers** Prior to prescribed (broadcast and pile) burning, or prior to use of heavy equipment to remove and/or masticate vegetation in badger denning habitat, which is characterized by herbaceous, shrub, and open stages of most habitats with dry, friable soils, a qualified wildlife biologist shall conduct a survey to identify any American badger burrows/dens. These surveys shall be conducted not more than 15 days prior to the start of work. American badger dens determined to be occupied during the breeding season (February 15 through June 30) shall be flagged, and ground-disturbing activities avoided within 100 feet to protect adults and nursing young. Buffers may be modified by the	

Impact Statement	Level of Significance before Mitigation	Mitigation Measures	Level of Significance after Mitigation
		qualified biologist, provided the badgers are protected, and shall not be removed until the qualified biologist has determined that the den is no longer in use. If the den is occupied during the non-maternity period (July 1 through February 14) and avoidance is not feasible, a passive badger relocation plan will be prepared and submitted to the CDFW for approval. Any passive relocation of American badgers shall occur only under the direction of a qualified biologist and with CDFW approval. **MM Biology-7: Protection of Nesting Birds** If mowing with heavy equipment or other vegetation (including tree) removal activities or prescribed (broadcast and pile) burning would commence anytime during the nesting/breeding season of native bird species (February 1 to September 1), a pre-construction survey for nesting birds shall be conducted by a qualified biologist within seven days of the habitat disturbance. The survey shall include visually surveying all suitable nesting habitat in the survey area, and be conducted during periods of high bird activity (i.e., 1-3 hours after sunrise and 1-3 hours before sunset). When the activity would occur along an existing fuel break or in other areas that are currently maintained such as along roads and in defensible spaces, then the survey area shall include only the disturbance footprint. During the construction of new fuelbreaks or during vegetation removal with heavy equipment in areas that were not previously managed (such as under MA-23 and MA-24), the survey area shall include the disturbance area and a surrounding buffer to be determined by a qualified biologist depending on type of equipment used, vegetation community, topography, resident bird species, and any other relevant factors. If active nests of bird species protected by the Migratory Bird Treaty Act and/or the California Fish and Game Code are found in areas that could be directly or indirectly disturbed (noise), a no-disturbance buffer zone shall be created around active nests during the breeding season or until a qualified biologist determines that all young have fledged. The size of the buffer zone shall be	

Impact Statement	Level of Significance before Mitigation	Mitigation Measures	Level of Significance after Mitigation
		determined by the biologist, by taking into account factors ~~such as~~ including but not limited to the following: 1. Noise and human disturbance levels at the site at the time of the survey and the noise and disturbance expected during the vegetation management activity; 2. Distance and amount of vegetation or other screening between the site and the nest; and 3. Sensitivity of individual nesting species and behaviors of the nesting birds. **MM Biology-8: Northern Spotted Owl Avoidance During Nesting Season** If mowing with heavy equipment, the mechanical removal of vegetation, or prescribed burning, including pile and broadcast burning, is to occur within the northern spotted owl nesting season (February 1 to July 31), the District shall commission two surveys for nesting northern spotted owls during the months of April and May preceding the commencement of these activities. At a minimum, the survey area shall include all suitable nesting habitats within 0.25 mile of any planned activity sites, and then one of the two options listed below shall be implemented: 1. Following a round of protocol-level northern spotted owl surveys in accordance with the USFWS *Protocol for Surveying Proposed Management Activities that may Impact Northern Spotted Owls* (USFWS, 2012), if it is conclusively determined that there are nesting northern spotted owls, planned activities that generate noise (e.g., mowing, heavy equipment usage) that are within 0.25-mile of an identified active nest shall not begin prior to September 1 unless the young have fledged, at which time work may begin no earlier than July 10. Prescribed burns may only occur within suitable northern spotted owl habitat (as determined by a qualified biologist) during the nesting season if protocol surveys have determined that northern spotted owl nesting is not occurring.	

Impact Statement	Level of Significance before Mitigation	Mitigation Measures	Level of Significance after Mitigation
		2. Alternatively, the District shall perform a calculation to determine the minimum buffer needed to avoid impacts on this species from noise generation by equipment. The calculation shall be based on the guidance and methodology in the USFWS "Transmittal of Guidance: Estimating the Effects of Auditory and Visual Disturbance to Northern Spotted Owls and Marbled Murrelets in Northwestern California," (USFWS, 2006) which takes into consideration the baseline noise levels, the noise and duration of noise generated by the loudest equipment, and the topography of the landscape. The resulting buffer calculated using these methods shall be a minimum buffer, but in no case shall the buffer be less than 500 feet. If the calculation is not performed, a conservative 0.25-mile buffer shall be implemented per (1), above. If nesting northern spotted owls are found, activities shall not occur prior to September 1 unless the young have fledged, at which time work may begin no earlier than July 10. Manual methods shall not occur within 131 feet of the line-of-site of a nesting northern spotted owl. **MM Biology-9: Protection of Western Pond Turtle Nesting and Overwintering Habitat** **Nesting** Any mechanical method of vegetation management that could crush turtle nests (i.e., heavy equipment), vehicle travel, or prescribed (broadcast and pile) burning that could occur where suitable western pond turtle nesting habitat is present shall be reviewed by a qualified biologist to determine if western pond turtle nesting could be present in the area. If the work with heavy equipment were to occur in loose soils in oak woodlands, mixed coniferous forests, broadleaf forests, or grasslands that are within 100 feet of ponds, during the western pond turtle egg-laying season (May to August) as determined by the qualified biologist, the activity shall either be rescheduled to occur outside of the egg-laying period; or a survey shall be conducted to determine if	

Impact Statement	Level of Significance before Mitigation	Mitigation Measures	Level of Significance after Mitigation
		eggs and nests are present in the work area and any identified eggs or nests and young turtles shall be avoided. **Overwintering of Hatchlings in Nests** Any mechanical method of vegetation management (i.e., heavy equipment) or vehicle travel that could occur where suitable overwintering habitat for hatchlings is present shall be reviewed by a qualified biologist to determine if any hatchlings could be present in the area. If work with heavy equipment were to occur in loose soils in oak woodlands, mixed coniferous forests, broadleaf forests, or grasslands that is within 225 meters of ponds known to be used by the western pond turtle, during the overwintering season (October to April) (Holland, 1994) as determined by the qualified biologist, the activity shall either be rescheduled to occur outside of the overwintering period, or a survey shall be conducted to determine if hatchlings are present in the work area and any identified nests shall be avoided. **MM Biology-10: California Red-Legged Frog Avoidance** Prior to implementing any vegetation management activities involving vehicles or equipment (i.e., mowers, graders, skid steer loader) within 0.25 mile of Lagunitas Creek downstream of Kent Lake, or around Soulajule Reservoir (or any location where California red-legged frogs have been found), a qualified biologist shall conduct protocol-level in accordance with the USFWS *Revised Guidance on Site Assessments and Field Surveys for the California Red-legged Frog* (USFWS, 2015) surveys the areas where activities are to occur to ensure that no California red-legged frogs are present in the activity footprint. The biologist shall also mark the work area and the maintenance crew shall be directed to stay within the marked activity areas. If California red-legged frogs are found, no work shall occur until the frogs have moved on their own from the activity area. **MM Biology-11: Marin Elfin Butterfly Host Plant Avoidance** Prior to vegetation management activities in the limited areas where stonecrop is known to occur (steep slopes on southeast	

Impact Statement	Level of Significance before Mitigation	Mitigation Measures	Level of Significance after Mitigation
		shore of Lake Lagunitas, north-facing slopes south of Alpine Lake, and north of Kent Lake), District botanical staff shall be notified. If the activity would occur in an area containing or potentially containing stonecrop, then a survey shall be conducted to flag all stonecrop plants within and bordering the work area. Work crews shall be instructed to avoid flagged plants or larger areas, and work crews shall be trained in identification of stonecrop. **MM Biology-12: Protection of Foot-Hill Yellow Legged Frog** Immediately prior to the use of heavy equipment, any other ground disturbing Plan activities, or prescribed (broadcast and pile) burning within 50 feet of Big Carson Creek, Little Carson Creek, or their tributaries, a clearance survey for foothill yellow-legged frog shall be conducted by an individual trained in the identification of the species. ~~Any identified foothill yellow-legged frogs shall be relocated (by a qualified biologist in possession of a valid Scientific Collecting Permit, or appropriate permit at the time of work if listing status changes) to a suitable location downstream of the activity area. Alternatively, the activity may be delayed until the frog has left the area on its own. Should the relocation of frogs be required, exclusionary fencing may be installed to prevent individual frogs from re-entering the activity area.~~ <u>If foothill yellow-legged frogs are found, no work shall occur until the frogs have moved on their own from the activity area.</u> **MM Biology-13: Mollusk Avoidance** Only hand methods of removal shall be used when working directly in seeps or springs, unless a survey for Marin Hesperian and robust walker is undertaken. If the species are not found in surveys, the work can proceed. If individuals are found, the area should be avoided or work shall only proceed using hand methods, supervised by a qualified biologist. If the use of equipment other than hand tools are required in Potrero Meadow, then a site-specific protection plan for Marin Hesperian and robust walker shall be prepared by a qualified biologist. The plan may include conducting clearance surveys	

Impact Statement	Level of Significance before Mitigation	Mitigation Measures	Level of Significance after Mitigation
		and having a qualified monitor onsite during construction activities, as well as ensuring that activities in that area would protect and/or enhance habitat in that area in the long-term. **MM Biology-14: Northern Spotted Owl ~~Avoidance of Nesting Season and Habitat~~** **Projects Within 0.25 Mile of an Activity Center** *Determine Type of Habitat Present* Prior to vegetation management within an area the latest GIS data available for northern spotted owl activity centers shall be consulted to determine whether the project is within 0.25 miles of an activity center. Once determined to be within 0.25 miles of an activity center, the habitat shall be reviewed to determine whether the project is proposed to occur within a forest habitat type that provides potential northern spotted owl foraging, roosting, and/or nesting habitat. This may be accomplished as follows: 1. A review of GIS data shall be conducted to determine if the activity is proposed to occur in a forest type potentially used by northern spotted owls (i.e., Douglas-fir, redwood, mixed conifer/hardwood forest, mature broadleaf/evergreen forest types). If the activity would not occur within a forest type potentially used by northern spotted owls, then no further actions is required to protect northern spotted owl habitat. 2. If the project is proposed to occur in a forest type potentially used by northern spotted owls, then a site-specific habitat evaluation shall be conducted within the month of February prior to the activity by a qualified northern spotted owl biologist to determine if the area provides the required habitat characteristics to provide northern spotted owl foraging, roosting, and/or nesting habitat. *Projects Within Appropriate Habitat*	

Impact Statement	Level of Significance before Mitigation	Mitigation Measures	Level of Significance after Mitigation
		For projects which are proposed to occur in potential northern spotted owl foraging, roosting, or nesting habitat, the following action shall be implemented prior to management activities: 3. Habitat alteration within core use areas (nesting and roosting habitat) shall be planned and conducted under the guidance of a qualified northern spotted owl biologist. Opportunities to conduct vegetation management to enhance development of late- successional characteristics or to meet other restoration goals in a manner compatible with retaining resident northern spotted owls shall be evaluated and implemented. Restoration activities conducted near northern spotted owl sites shall first focus on areas of younger forest less likely to be used by northern spotted owls and less likely to develop late-successional forest characteristics without vegetation management. Vegetation management projects shall be designed to include a mix of disturbed and undisturbed areas, retention of woody debris, and development of understory structural diversity to maintain small mammal populations across the landscape. 4. Presumed active woodrat stick nests (i.e., with visible signs of activity as determined by the qualified biologist) would be temporarily demarcated during surveys by the qualified biologist. Woodrat stick nests and areas around the nests, shall be avoided during vegetation ~~clearing~~ management activities. Any flagging or other markings would be removed following the activity. **MM Biology-17: Protection of California Giant Salamander** Immediately prior to the use of heavy equipment, any other ground disturbing Plan activities, or prescribed (pile and broadcast) burning within 50 feet of a stream or within riparian habitat, a clearance survey for California giant salamander shall be conducted by an individual trained in the identification of the species. Any identified California giant salamander shall be relocated (by a qualified biologist in possession of a valid Scientific Collecting Permit, or appropriate permit at the time of work if	

Impact Statement	Level of Significance before Mitigation	Mitigation Measures	Level of Significance after Mitigation
		listing status changes) to a suitable nearby location at least 250 feet from the original location. Alternatively, the activity may be delayed until the salamander has left the area on its own. **MM Geology-1: Erosion Control and Slope Stability Measures** Refer to Geology and Soils subheading below **MM Geology-3: Grazing Land and Trail Control** Refer to Geology and Soils subheading below **MM Hydrology-1: Water Quality Protection During Waterway Crossing or Work Near Waterbodies** Refer to Hydrology and Water Quality subheading below	

Table ES-1 on pages ES-52 through ES-54 has been revised as follows:

Impact Statement	Level of Significance before Mitigation	Mitigation Measures	Level of Significance after Mitigation
Geology and Soils			
Impact Geology and Soils-1: The proposed plan could result in substantial soil erosion or the loss of topsoil.	Potentially significant	**MM Geology-1: Erosion Control and Slope Stability Measures** Best management practices (BMPs) for forestry shall be implemented to ensure vegetation management does not result in erosion, loss of topsoil, or slope instability in areas where work could result in the exposure of bare soils or the loss of root-soil matrix strength. If groundcover is determined to be less than 70 percent[a] following work, then BMPs, as identified here, shall be implemented. Prior to conducting work in any given area under any management action that could result in erosion or slope instability (e.g., broadcast burns, tree removal, weed removal, or forest treatments that could reduce the groundcover and expose soil) the area shall be inspected for existing signs of erosion or slope instability (e.g. rills, slumped soil). Depending on the slope and the downslope resources (roads that could be impacted if a slope	Less than significant with mitigation

Impact Statement	Level of Significance before Mitigation	Mitigation Measures	Level of Significance after Mitigation
		failed, waterbodies or habitat that could be impacted from erosion, important habitat, etc.), erosion and slope stabilization measures shall be determined prior to implementation of work, based on the list below. Generally, if an action would expose soils (groundcover less than 70 percent), then measures to protect soils, minimize erosion, and prevent slope instability shall be implemented. The measures to be implemented shall depend on the site's specific characteristics and the type and extent of vegetation management work to be performed. The inspection and determination of appropriate measures shall be made by personnel with knowledge and experience in the application of erosion and slope stabilization BMPs through training or field experience with BMP installation. The personnel shall memorialize in writing their field observations, and corresponding recommendations regarding installation of BMPs. The following measures shall be implemented during work, if the activity would reduce groundcover by 70 percent or more and as applicable: • Minimize areas to be disturbed to the greatest extent feasible • Avoid use of heavy equipment on slopes greater than 30 percent • Shut down use of heavy equipment, skidding, and truck traffic when soils become saturated and unable to support the machines • Sow native grasses and other herbs on denuded areas where natural colonization or other replanting shall not occur rapidly; use slash or chips to prevent erosion on such areas • Use surface mounds, depressions, logs, rocks, trees and stumps, slash and brush, the litter layer, and native herbaceous vegetation downslope of denuded areas to reduce sedimentation and erosion, as necessary to prevent erosion or slope destabilization • Stabilize steep slopes (i.e., greater than 30 percent) with mats or natural materials after tree removal or weed removal and prior to planting, where soils are exposed and could erode • Broadcast burns shall be performed outside of perennial and intermittent streams, and riparian forest/woodland. A 50-foot	

Impact Statement	Level of Significance before Mitigation	Mitigation Measures	Level of Significance after Mitigation
		buffer around perennial and intermittent streams shall be maintained when the broadcast burn is proposed on a slope greater than 30 percent and upslope of the stream. • Install approved erosion control measures and non-filament-based geotextiles when: – conducting substantial ground disturbing work (i.e., use of heavy equipment, pulling large vegetation) within 100 feet[b] and upslope of currently flowing or wet wetlands, streams, lakes and riparian areas; – causing soil disturbance on moderate to steep (10 percent slope and greater) slopes; and – following the removal of invasive plants from stream banks to prevent sediment movement into watercourses and to protect bank stability • Sediment control devices, if installed, shall be certified weed-free, as appropriate. Sediment control devices shall be inspected daily to ensure that they are in good repair and working as needed to prevent sediment transport into the waterbodies (and repaired as needed) • Prior to conducting ground disturbing work the weather forecast shall be consulted; No substantial ground disturbing work (i.e., use of heavy equipment, pulling large vegetation) shall occur during rain events and 48 hours after a rain event, defined as 0.5 inch of rain or greater within a 48-hour ~~or greater~~ period, or until soils are determined to not be saturated. Once work is completed the areas shall be inspected as needed and as accessible but at least annually until groundcover exceeds 70 percent and it is clear that significant erosion and slope instability are not occurring. At that time, erosion control and slope stability devices shall be removed.	

3.2.2 Chapter 2 Project Description

Figure 2.4-2 on page 2-14 is revised as shown below.

Figures 2.6-1 through 2.6-4 on pages 2-20 through 2-23 are revised as shown below.

Page 2-36 is revised as follows:

> The target is to complete approximately 50 percent of the proposed fuelbreak expansion (~~59~~ 50 acres) within 5 years of BFFIP adoption.

Page 2-55 is revised as follows:

> Up to 84 workers could be conducting vegetation management activities on District lands on a single day, but generally, only a few crews for a total of up to 20 workers, would be operating simultaneously.

3.2.3 Section 3.3 Biological Resources

Table 3.3-5 on page 3.3-30 is revised as follows:

Scientific Name	Common Name	Listing Status Federal/State/Other			Habitat	Potential to Occur within District Lands
Pleuropogon hooverianus	North coast semaphore grass	-	ST	1B.1	Broadleaved upland forest, meadows and seeps, North Coast conifer forest understory, wet sites, grassy and sometimes shaded areas	Known to occur. Of the 4 documented Marin County sites, one population occurs in the Mt. Tamalpais watershed near Lagunitas Meadows but was presumed extirpated until re-discovery in 2019.

Table 3.3-6 on page 3.3-48 is revised as follows:

Scientific Name	Common Name	Listing Status Federal/State/Other			Habitat	Potential to Occur within District Lands
Rana boylii	Foothill yellow-legged frog	-	SPT/CSC	-	Foothill woodlands and chaparral near streams and ponds, riparian woodlands, wet meadows, also inhabits mixed conifer forest streams, slow streams and rivers with sunny, sandy and rocky or gravelly banks at 6,000 ft. and below in elevation.	Present in the Watershed and breeding in Little Carson Creek and Big Carson Creek. Also observed in Walker Creek, Cascade Creek, San Anselmo Creek, Carey Camp Creek, and Salmon Creek (downstream of Soulajule Reservoir).

Figure 2.4-2 Distribution of SOD in 2014

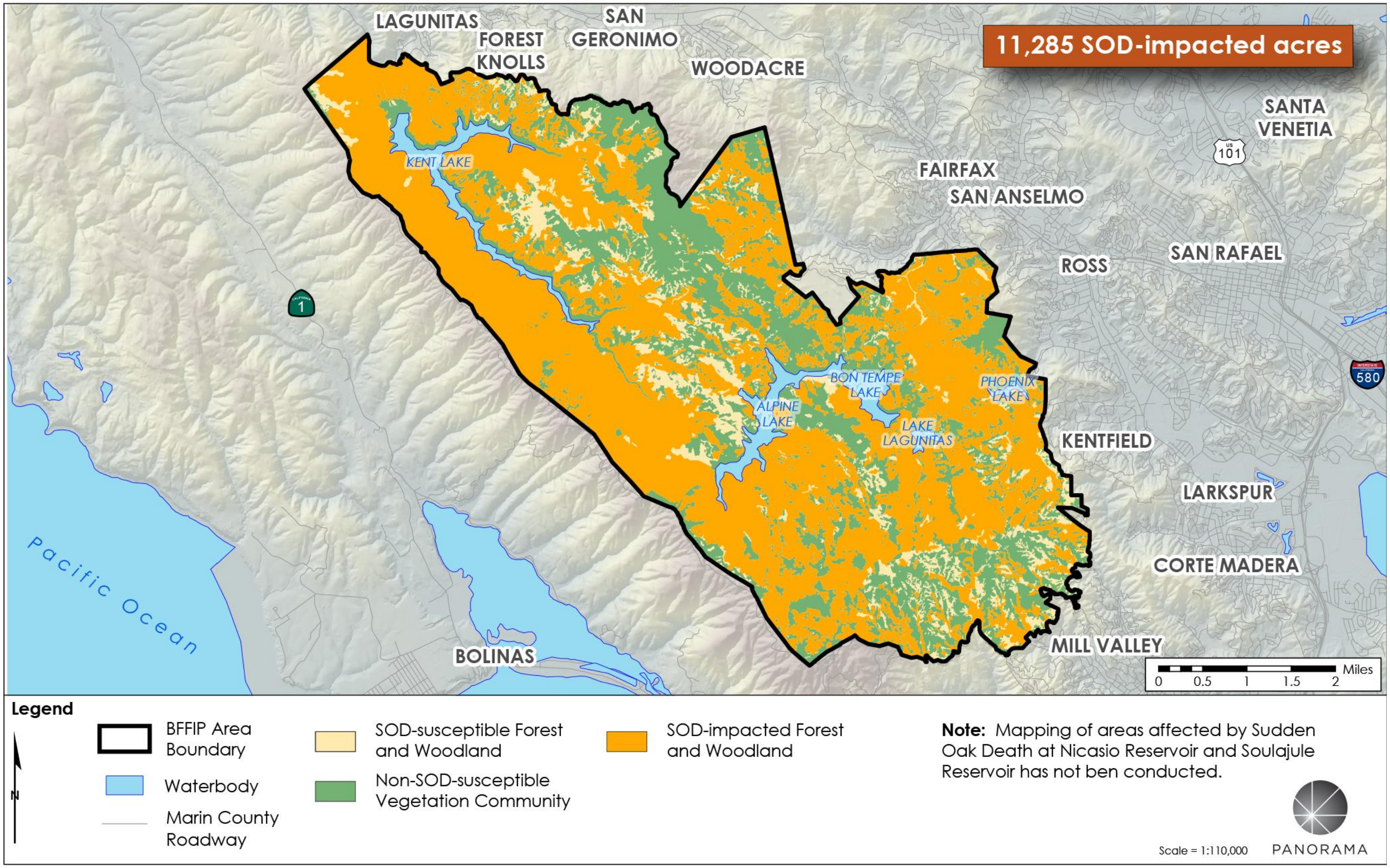


Figure 2.6-1 Infrastructure Zone (Map 1 of 5)

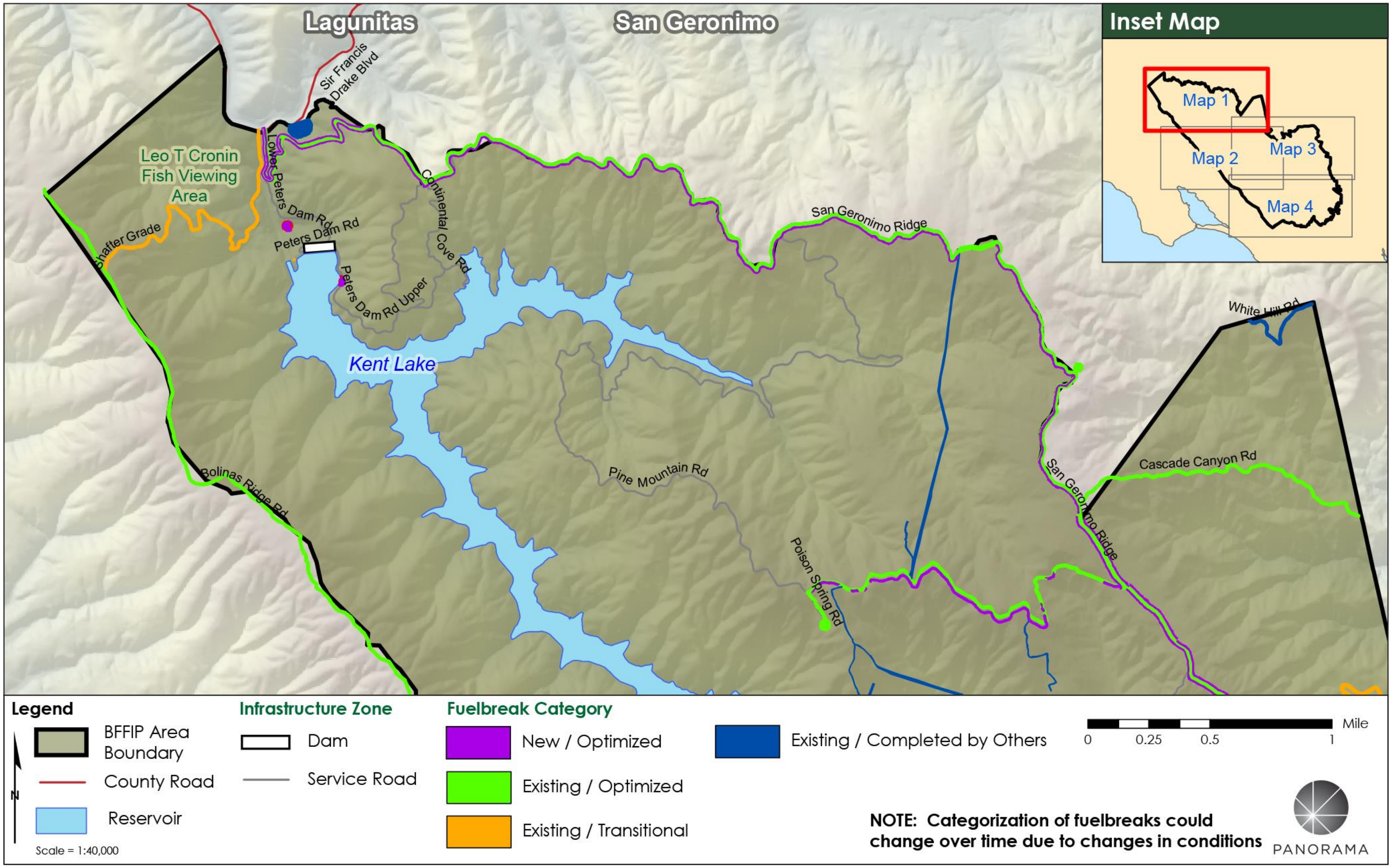


Figure 2.6-2 Infrastructure Zone (Map 2 of 5)

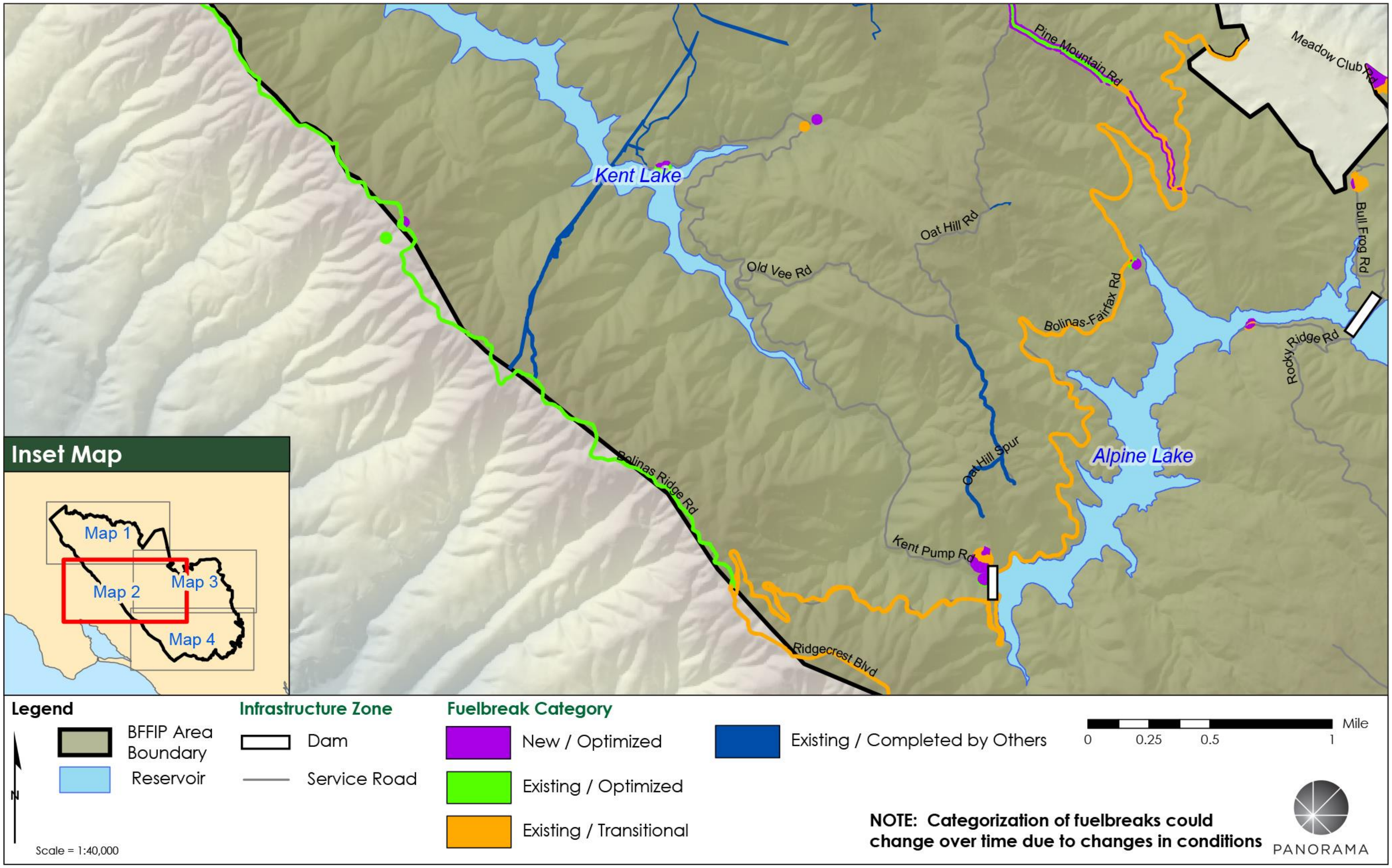


Figure 2.6-3 Infrastructure Zone (Map 3 of 5)

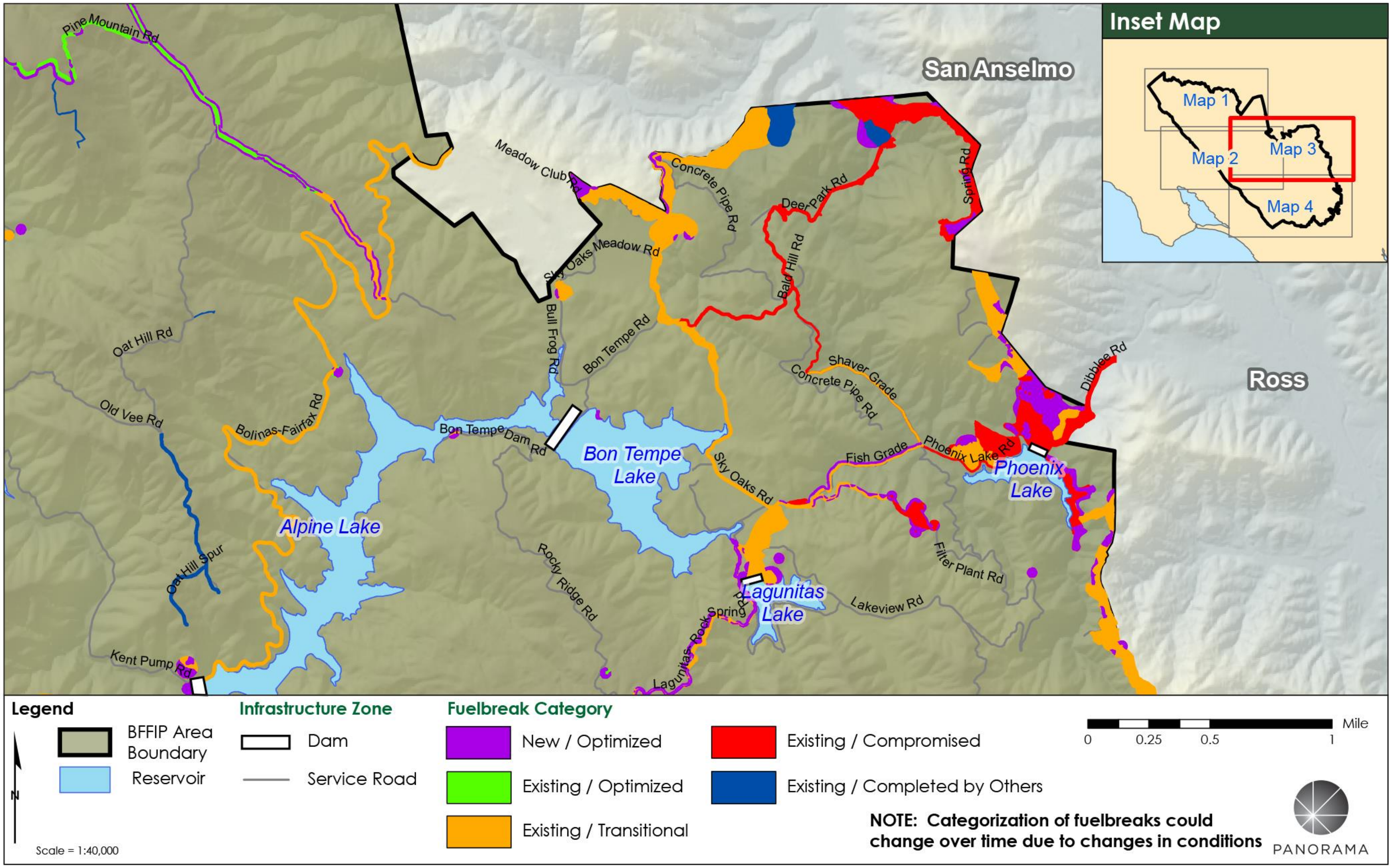
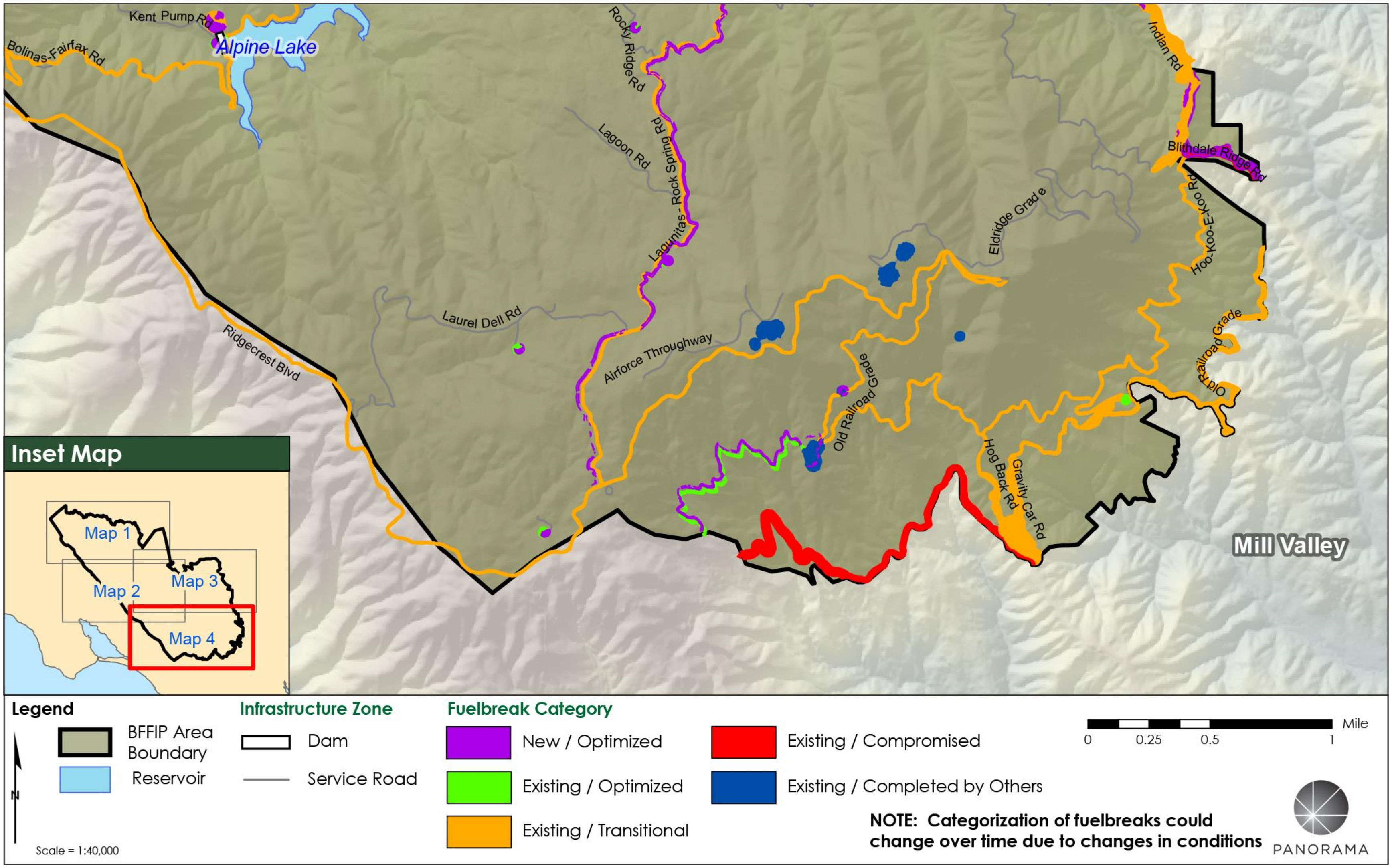


Figure 2.6-4 Infrastructure Zone (Map 4 of 5)



Page 3.3-58 is revised as follows:

During the 2016 survey of District lands and Marin County open space land, 42 sites were surveyed, of which 36 sites were occupied by pairs and 18 pairs attempted to nest.

All references to MM Biology-9 and MM Biology-14 in Section 3.3.6 are revised as follows:

MM Biology-9: Protection of Western Pond Turtle Nesting and Overwintering Habitat

MM Biology-14 (Northern Spotted Owl ~~Avoidance of Nesting Season and~~ Habitat)

Page 3.3-76 is revised as follows:

MM Geology-3 requires appropriate stocking of livestock in an area dependent upon applicable factors and ~~insuring~~ ensuring that overgrazing is not occurring and also prohibits grazing within 100 feet of waterways or waterbodies where special-status plants may grow.

Page 3.3-78 is revised as follows:

With implementation of mitigation, short-term, indirect impacts from habitat alteration on northern spotted owl would be less than significant.

Page 3.3-79 is revised as follows:

Prescribed pile burns would be limited in size and extent. Temporary smoke would be limited in extent and most piles would burn in a matter of a few hours. Impacts on bats from pile burning would be less than significant.

Low intensity burns, with shorter flame lengths (less than 1.6 meters) are unlikely to cause direct mortality or increase carbon monoxide levels to dangerous levels, since bats typically roost relatively high in tree canopies. Heat and smoke could still disturb bats roosting in trees, regardless of roost height (Perry, 2011). During times of lower ambient temperature, bats may not be able to rouse quickly enough to escape, or may expend too much energy escaping, placing them at risk of mortality. Broadcast burning could impact colonial and solitary roosting bats through the generation of smoke and heat from flames, were the burns to occur in the immediate vicinity of an individual roost, maternity roost, or bat colony. Impacts would be potentially significant.

Potentially Significant.

Page 3.3-82 is revised as follows:

Manual and mechanical methods of vegetation removal could occur in upland areas near reservoirs that are used for egg laying. ~~These~~ Mechanical activities could result in the loss of western pond turtle eggs or harm to individuals. The impact on western pond turtle would be potentially significant.

Page 3.3-88 is revised as follows:

> ~~Heavy equipment has~~ Manual and mechanical activities have the potential to crush stonecrop, the host plant for Marin elfin butterfly, or kill individual larvae or pupae. . Given the rarity of this species, the loss of individual larvae and stands of its host plant (stonecrop) would be a potentially significant impact.

Page 3.3-112 is revised as follows:

> **Planting**
> Preparation activities for planting would involve clearing of some vegetation by pulling or cutting. These activities could occur in or adjacent to sensitive plant communities but would be used to enhance the communities by planting rare plants that historically grew in the area. Plant species that are critical to a sensitive plant community could be accidentally removed or harmed. Equipment used to remove or trim vegetation could transmit forest pathogens to previously unaffected areas. The impact from loss of plants critical to a sensitive plant community would be potentially significant. BMP-4 through BMP-7 require the District to implement techniques to minimize the spread of forest diseases. The impact from forest diseases could remain significant. MM Biology-4 (Prevent the Spread of Forest Diseases from Plan Activities) ~~and MM Biology-16 (Protection of Native Grasslands)~~ would reduce the impacts on sensitive plant communities to less than significant.

MM Biology-2 on page 3.3-124 is revised as follows:

MM Biology-2: Protection of Special-Status Plants
The following measures shall be implemented to protect special-status plants: a. Prior to conducting any vegetation management activity (mechanical or manual removal), prescribed (broadcast and pile) burning, propane flaming, and animal grazing the area shall be reviewed by the District's botanist against the most current mapping data of special-status plant species and habitats. If the work is to occur in in serpentine habitat, within 500 feet of known special-status plant populations, near wetlands, or within other habitats with potential to support special-status plant populations, botanical surveys shall be conducted by a qualified botanist ahead of the planned work. The surveys shall be specific to the species of plants that could occur, must be conducted during a period when the special-status species that could occur in that habitat can be most readily detected (e.g. blooming period), and shall include the entire footprint of the proposed work. Any species identified during surveys shall be added to the GIS of current mapping data. ~~Areas only need to be surveyed within the previous 5 years.~~ If work is to occur again in the same area within 5 years (e.g., new fuelbreaks or retreatment areas for forestry actions), a new survey is not required. ~~b. For special-status species of low sensitivity ranking and that are common on District lands and resilient to disturbance (e.g., Mount Saint Helena morning-glory), disturbances shall be minimized to the degree practical but complete avoidance is not necessary, as directed by the MMWD botanist.~~ b. For listed species ~~of moderate or high sensitivity ranking,~~ with known rarity or declining populations including CRPR Rank 1B, 2, and some rank 4 species that are known rare), as determined and listed below by the MMWD botanical staff ~~(but not limited to this list)~~, the

MMWD's botanical staff shall ~~identify the appropriate avoidance measures to be implemented based on the life form~~:

i. Flag or otherwise demarcate the individual or population to ensure workers avoid the species for no loss of individuals.

ii. Establish a buffer of 100 feet around the individual or population.

iii. Require implementation of BMP-1 through BMP-3 for work conducted adjacent to these species to minimize the spread of invasive species.

- Brewer's milk vetch (*Astragalus breweri*)
- Brewer's calandrinia (*Calandrinia breweri*)
- Johnny-nip (*Castilleja ambigua var. ambigua*)
- Marin western flax (*Hesperolinon congestum*)
- Bristly leptosiphon (*Leptosiphon acicularis*)
- Santa Cruz microseris (*Stebbinsoseris decipiens*) *
- Coast rockcress (*Arabis blepharophylla*)
- Pink star-tulip (*Calochortus uniflorus*)
- Thin-lobed horkelia (*Horkelia tenuiloba*)
- Small groundcone (*Kopsiopsis hookeri*)
- Gairdner's yampah (*Perideridia gairdneri ssp. gairdneri*)
- North coast semaphore grass (*Pleuropogon hooverianus*)
- Marin manzanita (*Arctostaphylos virgata*)
- Glory brush (*Ceanothus gloriosus var. exaltatus*)
- Mason's ceanothus (*Ceanothus masonii*)

* This species is likely extirpated.

~~Species~~	~~Life Form~~
~~Mount Tamalpais oak (1B.3)~~	~~Perennial evergreen shrub~~
~~Mount Tamalpais manzanita (1B.3)~~	~~Perennial evergreen shrub~~
~~Marin manzanita (1B.2)~~	~~Perennial evergreen shrub~~
~~Glory brush (4.3)~~	~~Perennial evergreen shrub~~
~~Mason's ceanothus (SR, Rank 1B.2)~~	~~Perennial evergreen shrub~~
~~Western leatherwood (1B.2)~~	~~Perennial deciduous shrub~~
~~Napa false indigo (Rank 1B.2)~~	~~Perennial deciduous shrub~~
~~Serpentinite reed grass (4.3)~~	~~Perennial herb~~
~~Mount Tamalpais thistle (1B.2)~~	~~Perennial herb~~
~~California bottle-brush grass (4.3)~~	~~Perennial herb~~
~~Thin-lobed horkelia (1.B.2)~~	~~Perennial herb~~
~~Small groundcone (2B.3)~~	~~Perennial herb~~
~~Marsh zigadenus (Rank 4.2)~~	~~Perennial bulbiferous herb~~
~~Oakland star-tulip (4.2)~~	~~Perennial bulbiferous herb~~
~~Tiburon buckwheat (1B.2)~~	~~Annual herb~~
~~Marin western flax (FT, ST, Rank 1B.1)~~	~~Annual herb~~
~~Tamalpais lessingia (1B.2)~~	~~Annual herb~~
~~Marin County navarretia (Rank 1B.2)~~	~~Annual herb~~
~~Tamalpais jewel-flower (1B.3)~~	~~Annual herb~~

~~Mount Tamalpais bristly jewel-flower (1B.2)~~	~~Annual herb~~

c. For other listed species of CRPR rank 1B or 2 (beyond those identified in part b, above) with the potential to occur on District lands, the following measures shall be implemented:

i. Perennials:

1) Mark populations in the field with distinct flagging. Ensure that worker training is complete per MM Biology-1.

2) Avoid populations. If mowing cannot be safely performed up to the perimeter of the individuals, or timed for when they are senescent, then hand methods (i.e., hand pulling or use of non-powered or powered hand tools) shall be employed to prevent damage or removal of listed species.

3) Where tree or shrub species must be trimmed, such as Mount Tamalpais manzanita, follow any protocols or recommendations available, ~~such as~~ including the following the *Status and Management Recommendations for Arctostaphylos virgata (Marin Manzanita) in Point Reyes National Seashore* (Parker, 2007) and plant specific pruning tips (Las Pilitas Nursery, 2012) and perform the work by hand.

4) No net loss of an annual special-status species can occur. The population size shall be determined from the most recent survey data of the species.

If an individual or population must be removed, one or two options can be employed (subject to CDFW approval) and monitoring conducted to ensure that no net loss of the species occurs.

- (1) The individual or population can be dug up and relocated to appropriate habitat outside the work area. (2) A nursery with experience growing special-status plants can be employed to grow seedlings of the species that shall be planted in appropriate habitat outside the work area or in the work area following completion of work. If located outside the work area, appropriate habitat shall be within the same watershed as the impact area, and shall be identified or approved of by MMWD botanical staff.
- A monitoring plan shall be developed that details the following components. Conduct annual monitoring of seeded or replanted locations for a minimum of 3 years and up to 5 years, dependent upon the MMWD botanical staff recommendation and monitoring results. If the new population is not matching the pre-removal population data, more seeding or planting shall be conducted until pre-removal population is met.

ii. Annuals:

1) Flag or otherwise demarcate and ensure workers avoid the species as feasible; or,

2) Time vegetation management activities for when the special-status species occurring in the work area is senescent and/or after the seed has set.

3) Monitor populations between vegetation management activities to ensure that population sizes are not decreasing. If populations are decreasing and a correlation can be made to the maintenance activities, measures shall be identified by MMWD botanical staff and taken to improve the population, ~~such as~~ including but not limited to one of the following: avoiding the area in question or altering the management activity frequency.

4) No net loss of an annual special-status species can occur. The population size shall be determined from the most recent survey data of the species.
If an individual or population must be removed, one or two options can be employed and monitoring conducted to ensure that no net loss of the species occurs.

- (1) Seeds of the annuals shall be collected from existing on-site populations or from the same watershed (to maintain local genetic stock) and distributed in appropriate habitat outside the work area (within the same watershed) or in the work area following completion of work. (2) A nursery with experience growing special-status plants can be employed to grow seedlings of the species (from seeds collected locally) that shall be planted in appropriate habitat outside the work area or in the work area following completion of work. It should be noted that seeds derived from plants in the same watershed as the impact area may be available from local nurseries, and local nurseries may also be able to propagate seeds from adults grown from collected seeds. In this

	<u>case, seeds do not need to be collected from a specific impact area site. Appropriate habitat shall be identified or approved of by MMWD botanical staff.</u> • <u>A monitoring plan shall be developed that details the following components. Conduct annual monitoring of seeded or replanted locations for a minimum of 3 years and up to 5 years, dependent upon the MMWD botanical staff recommendation and monitoring results. If the new population is not matching the average population data, more seeding or planting shall be conducted until pre-removal population levels are met.</u>
Applicable Location(s): Serpentine habitat, within 500 feet of known special-status plant populations, near wetlands, or within other habitats with potential to support special-status plant populations	
Performance Standards and Timing: • **Before Activity:** (1) Check maps for habitat and known occurrences of special-status plants, (2) where applicable, conduct surveys in appropriate season (e.g. blooming season) before work is performed and record in GIS • **During Activity:** <u>(1)</u> Avoid ~~any~~ <u>the identified</u> special-status species<u>, (2) Avoid CRPR rank 1B and 2 special-status species or conduct reseeding/replanting</u> • **After Activity:** Monitor populations and make adjustment to future maintenance activities, if needed	

MM Biology-4 on page 3.3-126 is revised as follows:

MM Biology-4: Prevent the Spread of Forest Diseases from Plan Activities
Forest disease spread shall be evaluated by District biologists <u>when management actions are being performed.</u> ~~on an annual or more frequent basis, as dictated by the progression of the disease and the amount of habitat or vegetation impacted.~~ An evaluation shall be triggered when a District biologist observes that a native vegetation type within the BFFIP area has been impacted by the disease. The biologists shall determine if mechanical methods of vegetation removal could result in the spread of the disease in a given project area, prior to implementing the project. This evaluation shall be conducted by looking at the location of the disease, the types of species that are being impacted, and the methods by which the disease is spreading. If the disease is spread by soil contact, then the biologist shall prescribe methodologies for reducing spread from mechanical methods of vegetation management. These methods would likely be similar to those identified in BMP-4 through BMP-7 including, but not be limited to, washing equipment after working in infected areas, and planning work to progress from uninfected areas to infected areas.
Applicable Location(s): Where activities covering more than 5 acres could occur in areas of forest disease
Performance Standards and Timing: • **Before Activity:** Determine the areas where infestations are located and plan work accordingly to prevent spread • **During Activity:** Implement measures to prevent spread, such as by cleaning vehicles between work locations, if needed • **After Activity:** N/A

MM Biology-5 on page 3.3-126 is revised as follows:

MM Biology-5: Roosting Bats
<u>Broadcast Burning</u>

<u>Prior to conducting broadcast burning, a qualified biologist shall review the selected location to determine whether potential roosting bat habitat is present. If adequate roosting trees are present, one of two options may be pursued: (1) A qualified bat biologist shall first conduct a focused assessment of the roosting habitat within 2 days of burning to determine whether bats are present. If bats are present. the bat biologist shall determine whether the broadcast burn poses a threat to the roosting bats based on the location of the bats as compared with the prescribed burn location, wind directions, and type of fuel to be burned. If bats could be within direct line of smoke, a threat would occur If a threat could occur, the broadcast burn must be conducted when ambient temperatures are warmer to allow escape of the bats or the tree(s) avoided. (2) The broadcast burn will be conducted, avoiding the potential roosting trees.</u>

<u>Tree Removal</u>

Prior to the removal of trees with a DBH of greater than 10", a qualified biologist shall conduct a focused tree habitat assessment. Trees containing suitable potential bat roost habitat features shall be clearly marked or identified. If day roosts are found to be potentially present, the biologist shall prepare a site-specific roosting bat protection plan to be implemented. Based on site-specific conditions, the plan should incorporate the following guidance as appropriate:

Roost Avoidance

When possible, removal of trees identified as providing suitable roosting habitat should be conducted during seasonal periods of bat activity, including:

- Between March 1 and April 15, or after evening temperatures rise above 45 degrees Fahrenheit and/or no more than ½ inch of rainfall within 24 hours occurs; or
- Between September 1 and about October 15, or before evening temperatures fall below 45 degrees Fahrenheit and/or more than ½ inch of rainfall within 24 hours occurs.

If it is determined that a colonial maternity roost is potentially present, the roost shall be avoided and shall not be removed during the breeding season (April 15 to August 31) unless removal is necessary to address an imminent safety hazard.

Operation of mechanical equipment producing high noise levels (e.g., chainsaws, heavy equipment) in proximity to buildings/structures supporting or potentially supporting a colonial bat roost shall be restricted to periods of seasonal bat activity (as defined above), when possible.

Assessment

If work with loud, mechanical equipment must occur near a known or potential roosting structure/building during the maternity or hibernation roosting periods, then a qualified bat biologist shall first conduct a focused assessment of the structure. The site-specific plan shall be implemented to prevent noise-related impacts on roosting bats.

Roost Removal

If a tree potentially containing a colonial maternity roost must be removed, ~~such as~~ in the event of unsafe conditions requiring treatment, during the breeding season, then the following or other measures recommended by the qualified bat biologist may be implemented:

- Acoustic emergence surveys or other appropriate methods shall be conducted/implemented to further evaluate if the roost is an active maternity roost.
- If it is determined that the roost is not an active maternity roost, then the roost may be removed in accordance with the other requirements of this measure;
- If it is found that an active maternity roost of a colonial roosting species is present, the roost shall not be disturbed during the breeding season.

Potential colonial hibernation roosts will only be removed during seasonal periods of bat activity (i.e., non-hibernation periods). Potential non-colonial roosts that cannot be avoided shall be removed on warm days in late morning to afternoon when any bats present are likely to be warm and able to fly. Appropriate methods shall be used to minimize the potential of harm to bats during tree removal. Such methods may include using a two-step tree removal process. This method is conducted over two consecutive days, and works by creating noise and vibration by cutting non-habitat branches and limbs from habitat trees using chainsaws only (no excavators or other heavy machinery) on Day 1. The noise and vibration disturbance, together with the visible alteration of the tree, is very effective in causing bats

that emerge nightly to feed, to not return to the roost that night. The remainder of the tree is removed on Day 2.
Applicable Location(s): Where trees in bat roosting habitat could be impacted by activities (predominantly MA-21, MA-23, and MA-24)
Performance Standards and Timing: • **Before Activity:** (1) Conduct surveys if tree removal could occur in bat roosting areas and work is occurring during roosting, (2) humanely evict bats, if appropriate • **During Activity:** Avoid roosting bats • **After Activity:** N/A

MM Biology-7 on page 3.3-128 is revised as follows:

MM Biology-7: Protection of Nesting Birds
If mowing with heavy equipment or other vegetation (including tree) removal activities or prescribed (broadcast and pile) burning would commence anytime during the nesting/breeding season of native bird species (February 1 to September 1), a pre-construction survey for nesting birds shall be conducted by a qualified biologist within seven days of the habitat disturbance. The survey shall include visually surveying all suitable nesting habitat in the survey area, and be conducted during periods of high bird activity (i.e., 1-3 hours after sunrise and 1-3 hours before sunset). When the activity would occur along an existing fuelbreak or in other areas that are currently maintained such as along roads and in defensible spaces, then the survey area shall include only the disturbance footprint. During the construction of new fuelbreaks or during vegetation removal with heavy equipment in areas that were not previously managed (such as under MA-23 and MA-24), the survey area shall include the disturbance area and a surrounding buffer to be determined by a qualified biologist depending on type of equipment used, vegetation community, topography, resident bird species, and any other relevant factors. If active nests of bird species protected by the Migratory Bird Treaty Act and/or the California Fish and Game Code are found in areas that could be directly or indirectly disturbed (noise), a no-disturbance buffer zone shall be created around active nests during the breeding season or until a qualified biologist determines that all young have fledged. The size of the buffer zone shall be determined by the biologist, by taking into account factors ~~such as~~ <u>including but not limited to</u> the following: 5. Noise and human disturbance levels at the site at the time of the survey and the noise and disturbance expected during the vegetation management activity; 6. Distance and amount of vegetation or other screening between the site and the nest; and 7. Sensitivity of individual nesting species and behaviors of the nesting birds.
Applicable Location(s): Wherever heavy or noisy equipment is used to implement BFFIP management actions
Performance Standards and Timing: • **Before Activity:** (1) Conduct surveys, if appropriate, (2) identify nest buffers as needed • **During Activity:** Maintain non-disturbance areas around active nests. • **After Activity:** N/A

MM Biology-8 on page 3.3-129 is revised as follows:

MM Biology-8: Northern Spotted Owl Avoidance During Nesting Season
If mowing with heavy equipment, the mechanical removal of vegetation, or prescribed burning, including pile and broadcast burning, is to occur within the northern spotted owl nesting season (February 1 to July 31), the District shall commission two surveys for nesting northern spotted owls during the months of April and May preceding the commencement of these activities. At a minimum, the

survey area shall include all suitable nesting habitats within 0.25 miles of any planned activity sites, and then one of the two options listed below shall be implemented:

1. Following a round of protocol-level northern spotted owl surveys in accordance with the USFWS *Protocol for Surveying Proposed Management Activities that may Impact Northern Spotted Owls* (USFWS, 2012b), if it is conclusively determined that there are nesting northern spotted owls, planned activities that generate noise (e.g., mowing, heavy equipment usage) that are within 0.25-mile of an identified active nest shall not begin prior to September 1 unless the young have fledged, at which time work may begin no earlier than July 10. Prescribed burns may only occur within suitable northern spotted owl habitat (as determined by a qualified biologist) during the nesting season if protocol surveys have determined that northern spotted owl nesting is not occurring.
2. Alternatively, the District shall perform a calculation to determine the minimum buffer needed to avoid impacts on this species from noise generation by equipment. The calculation shall be based on the guidance and methodology in the USFWS "Transmittal of Guidance: Estimating the Effects of Auditory and Visual Disturbance to Northern Spotted Owls and Marbled Murrelets in Northwestern California," (USFWS, 2006) which takes into consideration the baseline noise levels, the noise and duration of noise generated by the loudest equipment, and the topography of the landscape. The resulting buffer calculated using these methods shall be a minimum buffer, but in no case shall the buffer be less than 500 feet. If the calculation is not performed, a conservative 0.25-mile buffer shall be implemented per (1), above. If nesting northern spotted owls are found, activities shall not occur prior to September 1 unless the young have fledged, at which time work may begin no earlier than July 10.

   Manual methods shall not occur within 131 feet of the line-of-site of a nesting northern spotted owl.

Applicable Location(s): Any areas of the District's lands where northern spotted owls can occur, including the Watershed and the Nicasio administrative unit

Performance Standards and Timing:

- **Before Activity:** (1) Conduct surveys, (2) as appropriate calculate buffer distances or conduct work outside of nesting season
- **During Activity:** Maintain buffers
- **After Activity:** N/A

MM Biology-9 on page 3.3-129 is revised as follows:

MM Biology-9: Protection of Western Pond Turtle Nesting <u>and Overwintering</u> Habitat

<u>Nesting</u>

Any mechanical method of vegetation management ~~that could crush turtle nests~~ (i.e., heavy equipment), vehicle travel, or prescribed (broadcast and pile) burning that could occur where suitable western pond turtle nesting habitat is present shall be reviewed by a qualified biologist to determine if western pond turtle nesting could be present in the area. If the work with heavy equipment were to occur in loose soils in oak woodlands, mixed coniferous forests, broadleaf forests, or grasslands that are within 100 feet of ponds, during the western pond turtle egg-laying season (May to August) as determined by the qualified biologist, the activity shall either be rescheduled to occur outside of the egg-laying period; or a survey shall be conducted to determine if eggs and nests are present in the work area and any identified eggs or nests and young turtles shall be avoided.

<u>Overwintering of Hatchlings in Nests</u>

<u>Any mechanical method of vegetation management (i.e., heavy equipment) or vehicle travel that could occur where suitable overwintering habitat for hatchlings is present shall be reviewed by a qualified biologist to determine if any hatchlings could be present in the area. If work with heavy equipment were to occur in loose soils in oak woodlands, mixed coniferous forests, broadleaf forests, or grasslands that is within 225 meters of ponds known to be used by the western pond turtle, during the</u>

overwintering season (October to April) (Holland, 1994) as determined by the qualified biologist, the activity shall either be rescheduled to occur outside of the overwintering period, or a survey shall be conducted to determine if hatchlings are present in the work area and any identified nests shall be avoided.

Applicable Location(s): Wherever heavy equipment, vehicle travel, or prescribed burning could occur in western pond turtle breeding habitat during their breeding season (May to August) or wherever heavy equipment and vehicle travel could occur during the overwintering season for hatchlings (October to April)

Performance Standards and Timing:

- **Before Activity:** (1) Biologist determines if the work area could support pond turtle breeding or overwintering based on the location of the work and proximity to ponds, (2) if no pond turtle could occur, work can proceed, (3) if pond turtle could be found in an area, the area shall be avoided or work rescheduled, (4) a survey can also be performed to rule out pond turtle eggs or overwintering hatchlings from the work area
- **During Activity:** Avoid pond turtle nests or overwintering hatchlings, if any had been found in surveys
- **After Activity:** N/A

MM Biology-12 on page 3.3-30 to 3.3-31 of the Draft EIR is revised as follows:

MM Biology-12: Protection of Foot-Hill Yellow Legged Frog

Immediately prior to the use of heavy equipment, any other ground disturbing Plan activities, or prescribed (broadcast and pile) burning within 50 feet of Big Carson Creek, Little Carson Creek, or their tributaries, a clearance survey for foothill yellow-legged frog shall be conducted by an individual trained in the identification of the species. ~~Any identified foothill yellow-legged frogs shall be relocated (by a qualified biologist in possession of a valid Scientific Collecting Permit, or appropriate permit at the time of work if listing status changes) to a suitable location downstream of the activity area. Alternatively, the activity may be delayed until the frog has left the area on its own. Should the relocation of frogs be required, exclusionary fencing may be installed to prevent individual frogs from re-entering the activity area.~~ If foothill yellow-legged frogs are found, no work shall occur until the frogs have moved on their own from the activity area.

Applicable Location(s): Activities (not including manual methods or planting) within 50 feet of Big Carson Creek, Little Carson Creek, or their tributaries

Performance Standards and Timing:

- **Before Activity:** (1) Survey for the species, ~~(2) move any individuals found in the work footprint prior to conducting activities~~
- **During Activity:** ~~N/A~~ If observed, activities must not occur until the individual(s) leave the area
- **After Activity:** N/A

MM Biology-14 on page 3.3-131 is revised as follows:

MM Biology-14: Northern Spotted Owl ~~Avoidance of Nesting Season and~~ Habitat

Projects Within 0.25 Mile of an Activity Center

Determine Type of Habitat Present

Prior to vegetation management within an area the latest GIS data available for northern spotted owl activity centers shall be consulted to determine whether the project is within 0.25 mile of an activity center. Once determined to be within 0.25 mile of an activity center, the habitat shall be reviewed to determine whether the project is proposed to occur within a forest habitat type that provides potential northern spotted owl foraging, roosting, and/or nesting habitat. This may be accomplished as follows:

1. A review of GIS data shall be conducted to determine if the activity is proposed to occur in a forest type potentially used by northern spotted owls (i.e., Douglas-fir, redwood, mixed

conifer/hardwood forest, mature broadleaf/evergreen forest types). If the activity would not occur within a forest type potentially used by northern spotted owls, then no further actions is required to protect northern spotted owl habitat.

2. If the project is proposed to occur in a forest type potentially used by northern spotted owls, then a site-specific habitat evaluation shall be conducted <u>within the month of February prior to the activity</u> by a qualified northern spotted owl biologist to determine if the area provides the required habitat characteristics to provide northern spotted owl foraging, roosting, and/or nesting habitat.

Projects Within Appropriate Habitat

For projects which are proposed to occur in potential northern spotted owl foraging, roosting, or nesting habitat, the following action shall be implemented prior to management activities:

1. Habitat alteration within core use areas (nesting and roosting habitat) shall be planned and conducted under the guidance of a qualified northern spotted owl biologist. Opportunities to conduct vegetation management to enhance development of late- successional characteristics or to meet other restoration goals in a manner compatible with retaining resident northern spotted owls shall be evaluated and implemented. Restoration activities conducted near northern spotted owl sites shall first focus on areas of younger forest less likely to be used by northern spotted owls and less likely to develop late-successional forest characteristics without vegetation management. Vegetation management projects shall be designed to include a mix of disturbed and undisturbed areas, retention of woody debris, and development of understory structural diversity to maintain small mammal populations across the landscape.
2. <u>Presumed active woodrat stick nests (i.e., with visible signs of activity as determined by the qualified biologist) would be temporarily demarcated during surveys by the qualified biologist.</u> Woodrat stick nests <u>and areas around the nests,</u> shall be avoided during vegetation ~~clearing~~ <u>management</u> activities. <u>Any flagging or other markings would be removed following the activity.</u>

Applicable Location(s): Areas within 0.25-miles of where northern spotted owls could forage, roost, or nest

Performance Standards and Timing:

- **Before Activity:** (1) Consult GIS layers to determine if a project would occur in northern spotted owl activity areas, (2) conduct surveys to evaluate habitat if work is to occur in a forest that could support northern spotted owls
- **During Activity:** Alter habitat as specified in measure, avoid woodrat stick nests
- **After Activity:** N/A

MM Biology-17 on page 3.3-133 is revised as follows:

MM Biology-17: Protection of California Giant Salamander

Immediately prior to the use of heavy equipment, any other ground disturbing Plan activities, or prescribed (pile and broadcast) burning within 50 feet of a stream or within riparian habitat, a clearance survey for California giant salamander shall be conducted by an individual trained in the identification of the species. Any identified California giant salamander shall be relocated (by a qualified biologist in possession of a valid Scientific Collecting Permit, or appropriate permit at the time of work if listing status changes) to a suitable nearby location <u>at least 250 feet from the original location</u>. Alternatively, the activity may be delayed until the salamander has left the area on its own.

Applicable Location(s): Activities (not including manual methods or planting) within 50 feet of a stream or within riparian habitat

Performance Standards and Timing:

- **Before Activity:** (1) Survey for the species, (2) move any individuals found in the work footprint prior to conducting activities
- **During Activity:** N/A
- **After Activity:** N/A

A new reference has been added under Section 3.3.8 References.

Dan C. Holland, P. (1994). *The Western Pond Turtle: Habitat and History.* Oregon Department of Fish and Wildlife.

Las Pilitas Nursery. (2012, December 30). *How to Prune Native Plants (without killing them).* Retrieved from Native Gardens: https://www.laspilitas.com/garden/howto/pruning.htm

Michl, Lisa. (2019, July 17). Marin County Parks Wildlife Biologist. (J. Schweitzer, Interviewer)

Perry, R. W. (2011). A Review Of Fire Effects On Bats And Bat Habitat In The Eastern Oak Region. *Proceedings of the 4th Fire in Eastern Oak Forests Conference*, (pp. 170-191).

3.2.4 Section 3.5 Geology and Soils

MM Geology-1 on page 3.5-38 has been revised as follows:

MM Geology-1: Erosion Control and Slope Stability Measures
Best management practices (BMPs) for forestry shall be implemented to ensure vegetation management does not result in erosion, loss of topsoil, or slope instability in areas where work could result in the exposure of bare soils or the loss of root-soil matrix strength. If groundcover is determined to be less than 70 percent[a] following work, then BMPs, as identified here, shall be implemented. Prior to conducting work in any given area under any management action that could result in erosion or slope instability (e.g., broadcast burns, tree removal, weed removal, or forest treatments that could reduce the groundcover and expose soil) the area shall be inspected for existing signs of erosion or slope instability (e.g. rills, slumped soil). Depending on the slope and the downslope resources (roads that could be impacted if a slope failed, waterbodies or habitat that could be impacted from erosion, important habitat, etc.), erosion and slope stabilization measures shall be determined prior to implementation of work, based on the list below. Generally, if an action would expose soils (groundcover less than 70 percent), then measures to protect soils, minimize erosion, and prevent slope instability shall be implemented. The measures to be implemented shall depend on the site's specific characteristics and the type and extent of vegetation management work to be performed. The inspection and determination of appropriate measures shall be made by personnel with knowledge and experience in the application of erosion and slope stabilization BMPs through training or field experience with BMP installation. The personnel shall memorialize in writing their field observations, and corresponding recommendations regarding installation of BMPs. The following measures shall be implemented during work, if the activity would reduce groundcover by 70 percent or more and as applicable: • Minimize areas to be disturbed to the greatest extent feasible • Avoid use of heavy equipment on slopes greater than 30 percent • Shut down use of heavy equipment, skidding, and truck traffic when soils become saturated and unable to support the machines • Sow native grasses and other herbs on denuded areas where natural colonization or other replanting shall not occur rapidly; use slash or chips to prevent erosion on such areas • Use surface mounds, depressions, logs, rocks, trees and stumps, slash and brush, the litter layer, and native herbaceous vegetation downslope of denuded areas to reduce sedimentation and erosion, as necessary to prevent erosion or slope destabilization

• Stabilize steep slopes (i.e., greater than 30 percent) with mats or natural materials after tree removal or weed removal and prior to planting, where soils are exposed and could erode • Broadcast burns shall be performed outside of perennial and intermittent streams, and riparian forest/woodland. A 50-foot buffer around perennial and intermittent streams shall be maintained when the broadcast burn is proposed on a slope greater than 30 percent and upslope of the stream. • Install approved erosion control measures and non-filament-based, biobased, biodegradable geotextiles when: – conducting substantial ground disturbing work (i.e., use of heavy equipment, pulling large vegetation) within 100 feet[b] and upslope of currently flowing or wet wetlands, streams, lakes and riparian areas; – causing soil disturbance on moderate to steep (10 percent slope and greater) slopes; and – following the removal of invasive plants from stream banks to prevent sediment movement into watercourses and to protect bank stability • Sediment control devices, if installed, shall be certified weed-free, as appropriate. Sediment control devices shall be inspected daily to ensure that they are in good repair and working as needed to prevent sediment transport into the waterbodies (and repaired as needed) • Prior to conducting ground disturbing work the weather forecast shall be consulted; No substantial ground disturbing work (i.e., use of heavy equipment, pulling large vegetation) shall occur during rain events and 48 hours after a rain event, defined as 0.5 inch of rain or greater within a 48-hour ~~or greater~~ period, or until soils are determined to not be saturated. Once work is completed the areas shall be inspected as needed and as accessible but at least annually until groundcover exceeds 70 percent and it is clear that significant erosion and slope instability are not occurring. At that time, erosion control and slope stability devices shall be removed.
Applicable Location(s): Any areas where the ground is disturbed and soils are exposed through vegetation management actions
Performance Standards and Timing: • **Before Activity:** Inspect areas for treatment prior to treatment to assess the potential for erosion and soil instability • **During Activity:** Implement the protection measures as needed to avoid or minimize erosion and slope instability • **After Activity:** Conduct inspections as needed after actions, depending on the size and nature of the work and the site, to ensure that erosion is not occurring and to remove any erosion control devices once they are no longer needed
Note: a Groundcover less than 70 percent has been found to result in excessive run-off and erosion (Lang & McDonald, 2005). b The 100-foot-buffer may be conservative but is based on literature reviews and studies that suggest a 100-foot-buffer is the adequate distance between streams and development to protect stream water quality, habitat, and organisms (Sweeney, 2014).

3.2.5 Section 3.11 Transportation

The analysis under Impact Transportation-1 on page 3.11-8 is revised as follows:

Impact Transportation-1: The proposed plan could conflict with or be inconsistent with CEQA Guidelines section 15064.3, subdivision (b).	**Significance Determination**
	Less than significant

A maximum of 84 workers could be conducting vegetation management activities on District lands on a single day. The likelihood of this occurring is quite low. ~~, but~~ g

Generally, only a few crews for a total of approximately 20 workers (40 one-way vehicle trips) would be operating simultaneously, at most, similar to existing conditions. The number of hours and days when work would occur would increase by approximately 300 percent compared to existing conditions. The annual average number of workers proposed under the BFFIP would increase from approximately one worker per day under existing conditions, to a maximum of 4 workers per day. Average daily, one-way vehicle trips throughout the year would increase from approximately 2 to 8 (or less). ~~Assuming a worst-case scenario that no workers carpool together, 84 vehicles trips per day could occur.~~ The net new, average daily number of one-way vehicle trips associated with the BFFIP ~~would~~ could increase nominally, but would not exceed 110 trips per day, the Office of Planning and Research's screening threshold, as previously discussed. The BFFIP would not conflict with State CEQA Guidelines section 15064.3, subdivision (b). The impact would be less than significant.

3.2.6 Chapter 4 Alternatives to the Proposed Plan

Page 4-31 is revised as follows:

The statement reflects the balancing of competing public objectives including factors such as environmental concerns, legal issues, technical, social, and economic factors.

3.2.7 Appendix G Cultural Resources Memo

Page 1 is revised as follows:

Vegetation management will also include weed control and utilize manual and mechanical techniques, and prescribed burning~~, and herbicides~~ for existing fuelbreak maintenance and defensible spaces. These actions may have temporary or permanent direct, indirect, and/or cumulative physical effects on both recorded and unknown cultural resources within the three administrative units.

Page 25 is revised as follows:

The MMWD plans to use various combinations of manual and mechanical techniques and prescribed burning to create fuelbreaks and defensible spaces depending on vegetation type (e.g., grasslands, shrublands, woodlands and forests). Vegetation management will also include weed control and utilize manual and mechanical techniques, and prescribed burning~~, and formulated organic herbicides (FOH) or conventional herbicides~~ for existing fuelbreak maintenance and defensible spaces.

Page 27 is revised as follows:

The proposed management and control actions including prescribed burning, removal via equipment ~~and herbicides~~, among others all possess the potential to change the known cultural resources.

4 MITIGATION MONITORING AND REPORTING PROGRAM

4.1 INTRODUCTION

When approving projects with mitigation measures that if implemented would avoid or lessen significant impacts, CEQA requires public agencies to adopt monitoring and reporting programs or conditions of project approval to mitigate or avoid the identified significant effects (Public Resources Code Section 21081.6(a)(1)). A public agency adopting measures to mitigate or avoid the significant impacts of a proposed project is required to ensure that the measures are fully enforceable, through permit conditions, agreements, or other means (Public Resources Code Section 21081.6(b)). The mitigation measures required by a public agency to reduce or avoid significant project impacts not incorporated into the design or program for the project may be made conditions of project approval as set forth in a Mitigation Monitoring and Reporting Program (MMRP), detailed in Table 4.3-1. The program must be designed to ensure project compliance with mitigation measures during project implementation. The District will use the Project Environmental Review Checklist, provided in Appendix A of this Final EIR, to evaluate if impacts of individual projects are covered in the Program EIR and to identify best management practices and mitigation measures that are applicable to those individual projects. Individual projects that do not conform to the scope of the Program EIR may require additional environmental analyses.

4.2 FORMAT

This MMRP is organized in a table format, keyed to each significant impact and mitigation measure.. Each mitigation measure is set out in full, followed by a tabular summary of monitoring requirements. The column headings in the tables are defined as follows:

- **Mitigation Measure.** This column presents the significant impact and full mitigation measure.
- **Implementation Responsibility.** This column assigns the party responsible for implementation of the measures
- **Monitoring Responsibility.** This column assigns the party responsible for monitoring implementation.
- **Timing and Performance Standards:** Identifies at which stage of the project, mitigation must be completed. Performance standards are identified that must occur during the specified stage of project implementation to determine that the objectives of the mitigation are met.

4.3 ENFORCEMENT

This MMRP will be incorporated as a condition of project approval. All mitigation measures must be carried out to fulfill the requirements of approval.

Table 4.3-1 Biodiversity, Fire, and Fuels Integrated Plan Mitigation, Monitoring, and Reporting Program

Best Management Practice and Mitigation Measure	Implementation Responsibility	Monitoring Responsibility	Applicable Locations	Timing and Performance Standards	Compliance Verification
Air Quality					
Impact Air-1 **MM Air-1: Broadcast Burn Emission Minimization Measures** Methods for reducing air pollutant emissions shall include one or more of the following: • Reducing the broadcast burn areas in each year. • When considering different types of prescribed burning projects, weigh the habitat benefits of burning in a particular fuel type against the emissions. With all other considerations being equal, choose lower emissions fuel types (such as grasslands versus hardwood or evergreen forest) for prescribed burning projects.	Contractor	The District	Where broadcast burns could occur.	**Before Activity:** (1) Reduce the acreage of broadcast burn, (2) Choose habitat types with fewer emissions, when other considerations are equal' (3) Reduce the fuel load in the forest understory **During Activity:** (1) Burn when the fuel has lower moisture, (2) Minimize fire duration **After Activity:** Quickly mop up	
Impact Air-2 **MM Air-2: Asbestos Management** Prior to conducting any activities requiring use of mechanical equipment (e.g., skid steer loader, backhoe) or off-road access of a project site, consult the map created using GIS that shows where serpentine soils and rock formations are located. If the project site or temporary access route passes through an area with serpentine soils or rock formations, implement the asbestos management measures (below). Prior to conducting any activities requiring manual soil-disturbing activities (e.g., pulling of small vegetation, planting seedlings), consult the GIS that shows where serpentine soils are located. If the project site is in an area with serpentine soils, implement the asbestos management measures (below). Asbestos Management Measures: • Areas known to have asbestos shall be watered during ground-disturbing activities (e.g., pulling of medium to large vegetation, digging large holes for planting) to ensure that the soil remains moist during the extent of the activity. • Vehicle speeds on unpaved roads shall be limited to 15 miles per hour. • When mowing in serpentine soils, the mower head shall be set at least 6 inches above the ground to minimize asbestos dust generation. If when mowing, dust is seen from the mower pluming more than 4 feet above the ground surface, the mower shall be adjusted to the minimum height needed to avoid generating dust plumes.	Contractor	The District	Areas with serpentine soils or rock formations where work could occur.	**Before Activity:** Water areas with serpentine soils or exposed rock formations **During Activity:** Limit vehicle speeds **After Activity:** N/A	
Impact Air-2 **MM Air-3: Minimization of Air Pollutant Risk** The District shall require that prescribed burns on its lands are conducted a minimum of 1,000 feet away from sensitive receptors, specifically residences, schools, and childcare centers. The District shall require that prescribed burns on its lands are managed to reduce District worker exposure to CO concentrations and other air pollutants through implementation of the following measures: • Use of realtime CO monitors • Rotate personnel out of heavy smoke areas • Avoid burning heavy fuel loads on the ground, such as large logs, to avoid additional mop-up • Tested and approved by NIOSH full-face and half-face air purifying respirators shall be equipped with filters for CO, formaldehyde, acrolein, and respirable particulate matter	Contractor	The District	Where broadcast and pile buns could occur.	**Before Activity:** (1) Purchase realtime CO monitors, (2) Purchase respirators and filters tested and approved by NIOSH **During Activity:** (1) Provide realtime CO monitor to firefighters, (2) Rotate firefighters out of heavy smoke areas, (3) Avoid burning of areas with heavy fuel loads, (4) Provide appropriate respirators and filters to firefighters **After Activity:** N/A	

<table>
<tr><th>Best Management Practice and Mitigation Measure</th><th>Implementation Responsibility</th><th>Monitoring Responsibility</th><th>Applicable Locations</th><th>Timing and Performance Standards</th><th>Compliance Verification</th></tr>
<tr><td>and available at all times for District staff or contractors working in the immediate vicinity of broadcast and pile burns</td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Impact Air-2
MM Air-4: Smoke Management Plan
Key considerations for broadcast and pile burns include, fuel, wind, relative humidity, air temperature, soil moisture, slope of the burn area, smoke management, and neighbouring land owners. A Smoke Management Plan and Prescribed Burn Plan (in accordance with MM Hazards-4) address the specifics related to these key factors. The District shall prepare a Smoke Management Plan in accordance with BAAQMD's Regulation 5 for all prescribed burns. The Smoke Management Plans shall be implemented for each burn. The Smoke Management Plan shall include all conditions and information detailed in Regulation 5, including the following:
• Burns shall not be ignited or fueled during calm conditions when winds are less than 5 miles per hour (mph) except for crossfiring, or when the wind direction at the site shall be such that the direction of smoke drift is toward a populated area in order to minimize local nuisances caused by smoke and particulate fallouts.
• Burns shall not be ignited or fueled when winds are more than 15 mph (NRCS, 2012).
• Burns shall not be ignited or fueled when wind direction blows towards populated areas.
• Identify the contingency actions that would be taken if a burn unexpectedly impacts sensitive receptors, identifiable by smoke complaints or presence of smoke in areas with receptors. Contingency actions include:
– halting ignition, suppressing fire, and/or beginning immediate mop up.</td><td>The District and Contractor</td><td>The District</td><td>Where broadcast and pile buns could occur.</td><td>Before Activity: Prepare a Smoke Management Plan including all identified details
During Activity: Implement the Smoke Management Plan
After Activity: N/A</td><td></td></tr>
<tr><td colspan="6">Impact Air-2: Implement Mitigation Measure MM Hazards-5 (see below)</td></tr>
<tr><td colspan="6">Impact Air-3: Implement Mitigation Measure MM Air-1 (see above)</td></tr>
<tr><td colspan="6">Impact Air-Cumulative: Implement Mitigation Measures MM Air-1, MM Air-2, and MM Air-3 (see above)</td></tr>
<tr><td colspan="6">Biological Resources</td></tr>
<tr><td>Impact Biology-1
BMP-1: Routine Operations and Project/Activity Implementation
District operations encompass a variety of management activities ranging from day-to-day road maintenance to Incident Command emergency situations. The following measures shall be implemented:
1. Prior planning may avoid the introduction and/or spread of weed species, such as by:
a. Implementing a periodic monitoring program for detecting new weed infestations in highly susceptible locations such as pull outs, railheads, picnic areas, parking lots, and concessionaire locations.
b. Defining "zero tolerance" zones in vulnerable, high-risk areas within the watershed which you commit to keeping weed-free through frequent monitoring and weed control efforts.
2. Minimize the extent and severity of soil disturbance, by:
a. Setting up staging areas and equipment in a way that will minimize soil disturbance and avoid loss of desirable native vegetation.
b. When working in vegetation types with relatively closed canopies, retaining shade to the extent possible to suppress weeds and prevent their establishment and growth.
3. Maintain facilities by implementing the following techniques:</td><td>The District and Contractor</td><td>The District</td><td>BFFIP Area</td><td>Before Activity: N/A
During Activity: (1) Avoid introduction and/or spread of weed species, (2) Minimize soil disturbance, (3) Maintain facilities
After Activity: N/A</td><td></td></tr>
</table>

Best Management Practice and Mitigation Measure	Implementation Responsibility	Monitoring Responsibility	Applicable Locations	Timing and Performance Standards	Compliance Verification
a. Maintain long-term staging areas, such as boneyards, dumps, and quarries in weed-free condition if possible, or contain weeds therein. If necessary, treat sites annually for weeds, and assign this duty to an appropriate, trained staff person. Consider ways of hardening these sites, such as deep mulching or scraping and tamping. b. Maintain trailheads, picnic areas, roads leading to trailheads, and other areas of concentrated public use in a weed-free condition. Make high-use recreation areas a high priority for weed detection and eradication if not already heavily infested.					
Impact Biology-1 **BMP-2: Pre-Work Assessments and Planning** Prevention begins with pre-work assessments and planning. The following are guidelines for general construction and maintenance activities: 1. Inspect all potential and current permitted activity sites. Incorporate invasive plant prevention and containment practices such as mowing, flagging or fencing invasive plant patches, designating invasive plant free travel routes and washing equipment. Where possible, avoid permitting activities that would result in the transfer of weed materials from an infested site to a non-infested site. Consider routes of travel, transport, and equipment use and address pathways and spread concerns with permittees. 2. Before ground-disturbing activities begin, inventory and prioritize weed infestations for treatment in construction sites and along access routes. Identify what weeds are on site or within the project's vicinity and do a risk assessment accordingly. Control these weed infestations. Ideally, weeds should be managed prior to the planned disturbance to minimize weed seeds in the soil. 3. Begin project operations in non-infested areas. Restrict movement of equipment or machinery from weed-contaminated areas to non-contaminated areas. 4. Locate and use weed-free project staging areas. Avoid or minimize travel through weed-infested areas, or restrict travel to those periods when spread of seed or propagules is least likely, such as prior to seed development.	The District and Contractor	The District	BFFIP Area	**Before Activity:** Conduct pre-work assessments and planning for construction and maintenance activities. **During Activity:** N/A **After Activity:** N/A	
Impact Biology-1 **BMP-3: Imports: Fills, Rock, Plant Material** Knowing the sources of imported material is critical to prevent the introduction of invasive plants. If a project involves moving plants or soil, consider the following: 1. Make sure plants and soil are not contaminated with weed seeds – use a certified weed free source or sterilize soil prior to use. 2. When possible, get the plants and soil from the worksite, which is less likely to introduce foreign material. 3. Inspect materials at the source to ensure that they are weed-free before transport and use. If sources of sand, gravel, and fill are infested, eradicate the weeds, then strip and stockpile the contaminated material for several years, if possible, to further deplete the soil seed bank. Check regularly for weed re-emergence and treat as needed. 4. Maintain stockpiled, non-infested material in a weed-free condition by preventing weed seed contamination with physical barriers and by frequently monitoring and quickly eradicating new weeds prior to seed production. 5. Use fill within the project area, or stockpile clean fill on-site for local use. Dispose of excess excavation or spoils in a way that won't spread weeds within the watershed or to neighbors.	Contractor	The District	BFFIP Area	**Before Activity:** Import weed-free plants and soil **During Activity:** (1) Maintain stockpile in weed-free condition, (2) Use native fill material, (3) Train staff to identify weeds and inventory weed infestations and schedule them for treatment **After Activity:** (1) Monitor construction sites with imported material annually for at least 3 years fate project completion, (2) Rehabilitate burn sites with seed and mulch, (3) Use native material to revegetate construction sites	

Best Management Practice and Mitigation Measure	Implementation Responsibility	Monitoring Responsibility	Applicable Locations	Timing and Performance Standards	Compliance Verification
6. Work with the weed specialist to develop guidelines for where earth materials can be moved within the watershed. 7. For routine purchase of material, such as rock used for drain or road base, work with the weed specialist to evaluate the risk, and if necessary develop a procedure for procuring weed-free material and/or inspecting materials sources. 8. Maintain stockpiled, non-infested material in a weed-free condition by preventing weed seed contamination with physical barriers (e.g. tarps) and by frequently monitoring and quickly eradicating new weeds prior to seed production. 9. Survey for, document, and treat weeds on construction sites (or wherever fill/material is brought in) annually for at least 3 years after project completion to ensure that any weeds transported to the site are promptly detected and eradicated. For on-going projects, continue to monitor until reasonably certain that weeds will not reappear. Plan for follow-up treatments based on inspection results. 10. Seed and mulch to be used for burn rehabilitation or slope stabilization (for wattles, straw bales, dams, etc.) all need to be inspected and certified that they are free of weed seed and propagules. Follow-up inspections of straw treated sites should be performed to insure any undetected source seed are treated. 11. Revegetation may include topsoil replacement, planting, seeding, and weed-free mulching as necessary. Use native material to the greatest extent possible. Consider stockpiling chipped local brush or cut and bale local weed-free grass for mulch – an added benefit is that mature seeds in the grass or brush can help restore local vegetation on the site. 12. Periodically inspect roads, trails, and rights-of-way for invasive plants. Train staff to recognize weeds and report locations to the local weed specialist. Inventory weed infestations and schedule them for treatment.					
Impact Biology-1 **BMP-4: Prevent Contamination of Clean Nursery Stock or other Clean Plant materials.** Planting stock shall be protected from potential contamination from the point that it leaves the production nursery or collection site until it has been planted. Note that container nursery stock has a high risk of infection by *Phytophthora* species if exposed to these pathogenic agents. Exclusion of these pathogens provides the only viable option for maintaining nursery plants free of *Phytophthora*. **Maintaining Nursery Stock in a Holding Facility** By definition, nursery stock produced by the District should be free of exotic *Phytophthora* to the maximum degree attainable. If such material is held for a period after delivery and before planting, the following clean nursery practices must be followed to prevent contamination of the nursery stock with *Phytophthora*: 1. Water used for irrigating plants shall comply with standards listed below. 2. Delivered nursery plants that will be held before planting shall be transferred to cleaned and sanitized raised benches and maintained as described below under Handling and Transporting Nursery Plants BMPs. **Handling and Transporting Nursery Plants** 3. Nursery plants shall be transported on or in vehicles or equipment that has been sanitized before loading the stock. Truck beds, racks, or other surfaces will be cleaned (swept, blown with compressed air and/or power washed as needed) to be free of soil and plant detritus. Cleaned surfaces shall be sanitized as described below under Procedures for Sanitizing Tools, Surfaces, and Footwear. 4. Keep plants in sanitized vehicles or on sanitized carts, trailers, etc. until delivered to their planting sites.	The District	The District	BFFIP Area	**Before Activity:** N/A **During Activity:** (1) Maintain nursery stock in a holding facility with cleaned and sanitized raised benches, (2) Transport nursery stock with sanitized vehicles or equipment, and place nursery stock clean waterproof surfaces, (3) Use clean water sources for washing, soaking, or irrigation, (4) Use pre-approved materials for mulch, compost, and soil amendment, and inoculants, (5) Use new and uncontaminated irrigation supplies, erosion control fabrics, fencing, stakes, posts, and other planting site inputs **After Activity:** N/A	

Best Management Practice and Mitigation Measure	Implementation Responsibility	Monitoring Responsibility	Applicable Locations	Timing and Performance Standards	Compliance Verification
5. At the job site, plants shall be handled to prevent contamination until delivered to each planting site. Nursery stock shall not be staged on the soil or other potentially contaminated surfaces except that plants may be placed on the soil surface at their specific planting sites. 6. If it is necessary to offload plants at the job site, plants may be placed on clean waterproof plastic tarps or other clean, sanitized surfaces. If tarps are used for holding plants, one surface will be dedicated for contact with nursery stock and will be cleaned and sanitized as needed to maintain phytosanitary conditions. **Other Planting Site Inputs** 7. Washing, soaking, or irrigation of plant material shall be conducted using clean water sources as specified below under Clean Water Specifications. Untreated surface waters shall not be used for these purposes. 8. Mulch, compost, soil amendments, inoculants, and other organic products shall be pre-approved for use before delivery to the planting site. Materials shall be free of pathogen contamination due to composition, manufacturing conditions, or through effective heat treatment and subsequently handled and maintained in a manner to prevent contamination. If appropriate, testing may be required as specified by the District. At the job site, delivered materials shall be handled to prevent contamination until delivered to each planting site in the same manner specified above under Handling and Transporting Nursery Plants. 9. All other materials to be installed at the site shall be of new material that has not been stored in contact with soil, untreated surface waters, or other potentially contaminated materials. This includes irrigation supplies (such as pipe, fittings, valves, drip line, emitters, etc.), erosion control fabrics, fencing, stakes, posts, and other planting site inputs.					
Impact Biology-1 **BMP-5: Cleaning and Sanitation Required Before Entering Planting Area to Prevent Introducing Contamination from Other Locations** *Phytophthora* contamination can be present in agricultural and landscaped areas, in commercial nursery stock, and in some infested native or restored habitat areas. Contamination can be spread via soil, plant material and debris, and water from infested areas. Arriving at the site with clean vehicles, equipment, tools, footwear, and clothing helps prevent unintentional contamination of the planting site from outside sources. **Vehicles, Equipment, and Tools** 1. Equipment, vehicles and large tools must be free of soil and debris on tires, wheel wells, vehicle undercarriages, and other surfaces before arriving at the planting area. A high pressure washer and/or compressed air may be used to ensure that soil and debris are completely removed. Vehicles that only travel and park on paved roads do not require external cleaning. 2. Contractors will comply with this provision by demonstrating that the equipment has been cleaned at a commercial vehicle or appropriate truck washing facility 3. The interior of equipment (cabs, etc.) must be free of mud, soil, gravel and other debris. Interiors may be vacuumed or washed. 4. Small tools and other small equipment (including hoses, quick couplers, hose nozzles, and irrigation wands) must be washed to be free of soil or other contamination and sanitized as described below in Procedures for Sanitizing Tools, Surfaces, and Footwear. 5. Hoses shall be new or previously used only for clean water sources as described below in Clean Water Specifications. **Footwear and Clothing**	The District and Contractor	The District	BFFIP Area	**Before Activity:** Clean and sanitize vehicles, equipment, tools, footwear, and clothing before entering planting areas **During Activity:** N/A **After Activity:** N/A	

Best Management Practice and Mitigation Measure	Implementation Responsibility	Monitoring Responsibility	Applicable Locations	Timing and Performance Standards	Compliance Verification
6. Soles and uppers of footwear must be free of debris and soil before arriving at the planting area. Clean and sanitize footwear as described in Procedures for Sanitizing Tools, Surfaces, and Footwear. 7. At the start of work at each new job site, worker clothing shall be free of all mud, soil or detritus. If clothing is not freshly laundered, all debris and adhered soil should be removed by brushing with a stiff brush.					
Impact Biology-1 **BMP-6: Prevent Potential Spread of Contamination within Planting Areas** *Phytophthora* can also be spread within plantings areas if some portions of the site are contaminated. However, it is not possible to identify every portion of a planting area that contains or is free of *Phytophthora*. Because *Phytophthora* contamination is not visible, working practices should minimize the movement of soil within the planting area to minimize the likelihood of spreading contamination. The District may designate specific portions of a planting area as having high or low risk of contamination. Areas with higher risk of contamination typically include areas adjacent to planted landscaping, areas previously planted with *Phytophthora*-infected stock, areas with existing or recently removed woody vegetation, areas directly along watercourses. Areas with low risk of contamination typically include upland sites with only grassy vegetation or sites where surface soils have been removed. **Worker Training and Site Access** 1. Before entering the job site, field workers and contractors shall receive training that includes information on *Phytophthora* diseases and how to prevent the spread of these and other soil borne pathogens by following approved phytosanitary procedures. 2. Do not bring more vehicles into the planting area than absolutely necessary. Within the planting area, keep vehicles on surfaced or graveled roads whenever possible to minimize potential for soil movement. 3. Travel off roads or on unsurfaced roads should be avoided when such roads are wet enough that soil will stick to vehicle tires and undercarriages. **Especially from Higher to Lower Risk Areas** 4. Brush off substantial soil contamination from tools and gloves when moving between successive planting sites to prevent repeated collection and deposition of soil across multiple sites. 5. Avoid contaminating clothing with soil during planting operations. Use nonporous knee pads that are cleaned between planting sites if kneeling is necessary. 6. When possible, plant nursery stock from a given block in the same local area rather than spreading it widely. If a problem is associated with a given block of plants, it will be easier to detect and deal with it if the plants are spatially grouped. 7. Phase work to minimize movement between areas with high and low risk of contamination. Where possible, complete work in low risk areas before moving to higher risk areas. Alternatively, restrict personnel to working in either high or low risk areas exclusively to reduce the need for decontamination. 8. Clean soil and plant debris from large equipment and sanitize hand tools, buckets, gloves, and footwear when moving from higher risk to lower risk areas or when moving between widely separated portions of the planting area. 9. All non-plant materials to be installed at the site (irrigation equipment, erosion control fabric, fencing, etc.) shall be handled to prevent movement of soil within the site, especially movement from higher risk to lower risk areas. Materials should be kept free of soil contamination by maintaining them in sanitized vehicles or on sanitized carts, trailers, etc., or stockpiling in elevated dry areas on clean tarps until used.	The District and Contractor	The District	BFFIP Area	**Before Activity:** (1) Any staff, contractors or volunteers performing any work in planting areas shall receive training about *Phytophthora* diseases and other soil borne pathogens, (2) Designate high and low risk contamination areas **During Activity:** (1) Avoid travelling on wet off roads or unsurfaced roads, (2) Clean and sanitize footwear and clothing when moving from higher to lower risk areas, (3) Keep all non-plant materials free of soil contamination **After Activity:** N/A	

Best Management Practice and Mitigation Measure	Implementation Responsibility	Monitoring Responsibility	Applicable Locations	Timing and Performance Standards	Compliance Verification
Impact Biology-1 **BMP-7: Procedures for Sanitizing Tools, Surfaces, and Footwear** Surfaces and tools should be clean and sanitized before use. Tools and working surfaces (e.g., potting benches) should be smooth and nonporous to facilitate cleaning and sanitation. Wood handles on tools should be sealed with a waterproof coating to make them easier to sanitize. Before sanitizing, removal all soil and organic material (roots, sap, etc.) from the surface. If necessary, use a detergent solution and brush to scrub off surface contaminants. The sanitizing agent may also be used as a cleaning fluid. Screwdrivers or similar implements may be needed to clean soil out of crevices or shoe treads. Brushes and other implements used to help remove soil must be cleaned and sanitized after use.	The District and Contractor	The District	BFFIP Area	**Before Activity:** Clean and sanitize tools, surfaces, and footwear prior to working in planting areas **During Activity:** N/A **After Activity:** N/A	
Impact Biology-1 **MM Biology-1: Worker Training** An environmental training program shall be developed and presented by a qualified biologist to all vegetation management workers before they are allowed to perform work under the BFFIP. The training shall describe special-status species and sensitive habitats that could occur within vegetation management areas, protection afforded these species and habitats, and the avoidance and minimization measures required to avoid and/or minimize impacts on these species and habitats, including maintaining avoidance areas, identification of species for avoidance, and protocols to follow, including protocols for minimizing the spread of invasive species and forest diseases.	Contractor working with qualified biologist	The District	BFFIP Area.	**Before Activity:** (1) This measure would be implemented prior to any staff, contractors or volunteers performing any work under the plan, (2) sign-in sheets for trained staff should be maintained by District staff **During Activity:** N/A **After Activity:** N/A	
Impact Biology-1 **MM Biology-2: Protection of Special-Status Plants** The following measures shall be implemented to protect special-status plants: a. Prior to conducting any vegetation management activity (mechanical or manual removal), prescribed (broadcast and pile) burning, propane flaming, and animal grazing the area shall be reviewed by the District's botanist against the most current mapping data of special-status plant species and habitats. If the work is to occur in in serpentine habitat, within 500 feet of known special-status plant populations, near wetlands, or within other habitats with potential to support special-status plant populations, botanical surveys shall be conducted by a qualified botanist ahead of the planned work. The surveys shall be specific to the species of plants that could occur, must be conducted during a period when the special-status species that could occur in that habitat can be most readily detected (e.g. blooming period), and shall include the entire footprint of the proposed work. Any species identified during surveys shall be added to the GIS of current mapping data. If work is to occur again in the same area within 5 years (e.g., new fuelbreaks or retreatment areas for forestry actions), a new survey is not required. b. For listed species with known rarity or declining populations including CRPR Rank 1B, 2, and some rank 4 species that are known rare), as determined and listed below by the MMWD botanical staff~~,~~ the MMWD's botanical staff shall: i. Flag or otherwise demarcate the individual or population to ensure workers avoid the species for no loss of individuals. ii. Establish a buffer of 100 feet around the individual or population. iii. Require implementation of BMP-1 through BMP-3 for work conducted adjacent to these species to minimize the spread of invasive species. • Brewer's milk vetch (*Astragalus breweri*) • Thin-lobed horkelia (*Horkelia tenuiloba*) • Brewer's calandrinia (*Calandrinia breweri*) • Small groundcone (*Kopsiopsis hookeri*)	The District's botanist and Contractor	The District	Serpentine habitat, within 500 feet of known special-status plant populations, near wetlands, or within other habitats with potential to support special-status plant populations.	**Before Activity:** (1) Check maps for habitat and known occurrences of special-status plants, (2) where applicable, conduct surveys in appropriate season (e.g. blooming season) before work is performed and record in GIS. **During Activity:** (1) Avoid the identified special-status species, (2) Avoid CRPR rank 1B and 2 special-status species or conduct reseeding/replanting **After Activity:** Monitor populations and make adjustment to future maintenance activities, if need.	

Best Management Practice and Mitigation Measure	Implementation Responsibility	Monitoring Responsibility	Applicable Locations	Timing and Performance Standards	Compliance Verification
• Johnny-nip (*Castilleja ambigua var. ambigua*) • Marin western flax (*Hesperolinon congestum*) • Bristly leptosiphon (*Leptosiphon acicularis*) • Santa Cruz microseris (*Stebbinsoseris decipiens*) * • Coast rockcress (*Arabis blepharophylla*) • Pink star-tulip (*Calochortus uniflorus*) • Gairdner's yampah (*Perideridia gairdneri ssp. gairdneri*) • North coast semaphore grass (*Pleuropogon hooverianus*) • Marin manzanita (*Arctostaphylos virgata*) • Glory brush (*Ceanothus gloriosus var. exaltatus*) • Mason's ceanothus (*Ceanothus masonii*) * This species is likely extirpated c. For other listed species of CRPR rank 1B or 2 (beyond those identified in part b, above) with the potential to occur on District lands, the following measures shall be implemented: i. Perennials: 1) Mark populations in the field with distinct flagging. Ensure that worker training is complete per MM Biology-1. 2) Avoid populations. If mowing cannot be safely performed up to the perimeter of the individuals, or timed for when they are senescent, then hand methods (i.e., hand pulling or use of non-powered or powered hand tools) shall be employed to prevent damage or removal of listed species. 3) Where tree or shrub species must be trimmed, such as Mount Tamalpais manzanita, follow any protocols or recommendations available, ~~such as~~ including the following the *Status and Management Recommendations for Arctostaphylos virgata (Marin Manzanita) in Point Reyes National Seashore* (Parker, 2007) and plant specific pruning tips (Las Pilitas Nursery, 2012) and perform the work by hand. 4) No net loss of an annual special-status species can occur. The population size shall be determined from the most recent survey data of the species. If an individual or population must be removed, one or two options can be employed (subject to CDFW approval) and monitoring conducted to ensure that no net loss of the species occurs. • (1) The individual or population can be dug up and relocated to appropriate habitat outside the work area. (2) A nursery with experience growing special-status plants can be employed to grow seedlings of the species that shall be planted in appropriate habitat outside the work area or in the work area following completion of work. If located outside the work area, appropriate habitat shall be within the same watershed as the impact area, and shall be identified or approved of by MMWD botanical staff. • A monitoring plan shall be developed that details the following components. Conduct annual monitoring of seeded or replanted locations for a minimum of 3 years and up to 5 years, dependent upon the MMWD botanical staff recommendation and monitoring results. If the new population is not matching the pre-removal population data, more seeding or planting shall be conducted until pre-removal population is met. ii. Annuals: 1) Flag or otherwise demarcate and ensure workers avoid the species as feasible; or,					

Best Management Practice and Mitigation Measure	Implementation Responsibility	Monitoring Responsibility	Applicable Locations	Timing and Performance Standards	Compliance Verification
2) Time vegetation management activities for when the special-status species occurring in the work area is senescent and/or after the seed has set. 3) Monitor populations between vegetation management activities to ensure that population sizes are not decreasing. If populations are decreasing and a correlation can be made to the maintenance activities, measures shall be identified by MMWD botanical staff and taken to improve the population, including but not limited to one of the following: avoiding the area in question or altering the management activity frequency. 4) No net loss of an annual special-status species can occur. Due to the variations in population from year to year as a result of weather fluctuations, average population data can be calculated from several years of data collected during the annual census conducted by MMWD or by volunteers as directed by MMWD. 5) If an individual or population must be removed, one or two options can be employed and monitoring conducted to ensure that no net loss of the species occurs. • (1) Seeds of the annuals shall be collected from existing on-site populations or from the same watershed (to maintain local genetic stock) and distributed in appropriate habitat outside the work area (within the same watershed) or in the work area following completion of work. (2) A nursery with experience growing special-status plants can be employed to grow seedlings of the species (from seeds collected locally) that shall be planted in appropriate habitat outside the work area or in the work area following completion of work. It should be noted that seeds derived from plants in the same watershed as the impact area may be available from local nurseries, and local nurseries may also be able to propagate seeds from adults grown from collected seeds. In this case, seeds do not need to be collected from a specific impact area site. Appropriate habitat shall be identified or approved of by MMWD botanical staff. • A monitoring plan shall be developed that details the following components. Conduct annual monitoring of seeded or replanted locations for a minimum of 3 years and up to 5 years, dependent upon the MMWD botanical staff recommendation and monitoring results. If the new population is not matching the average population data, more seeding or planting shall be conducted until pre-removal population levels are met.					
Impact Biology-1 **MM Biology-3: Prevent the Spread of Invasive Species** Precautions shall be taken to minimize the introduction of any invasive weeds or to prevent the spread of existing infestations. Prior to conducting an activity that requires the use of mechanical equipment; the area shall be reviewed by a qualified biologist against the most recent maps of invasive species infestation. The biologist shall direct the work crews as to the need for vehicle cleaning and/or the order in which work should be conducted to minimize the possible spread of invasive species. If work is to commence in an area of known invasive species infestation, the work shall be limited to the area of infestation and no equipment shall move to uninfested areas without being washed first. Alternatively, work shall start in the uninfested areas and progress to the more heavily infested areas last. Areas of broadcast burns shall be monitored annually to ensure that invasive species/weeds are not taking over. Invasive species shall be removed until native vegetation establishes.	Contractor working with qualified biologist	The District	Where activities covering more than 5 acres could occur in areas of invasive species.	**Before Activity:** Determine the areas where infestations are located and plan work accordingly to prevent spread **During Activity:** Clean vehicles between locations, if needed **After Activity:** Monitor burn areas for invasive species and weeds	
Impact Biology-1 **MM Biology-4: Prevent the Spread of Forest Diseases from Plan Activities**	Contractor working with the District's biologists	The District	Where activities covering more than 5 acres could occur in areas of forest disease	**Before Activity:** Determine the areas where infestations are	

Best Management Practice and Mitigation Measure	Implementation Responsibility	Monitoring Responsibility	Applicable Locations	Timing and Performance Standards	Compliance Verification
Forest disease spread shall be evaluated by District biologists when management actions are being performed. An evaluation shall be triggered when a District biologist observes that a native vegetation type within the BFFIP area has been impacted by the disease. The biologists shall determine if mechanical methods of vegetation removal could result in the spread of the disease in a given project area, prior to implementing the project. This evaluation shall be conducted by looking at the location of the disease, the types of species that are being impacted, and the methods by which the disease is spreading. If the disease is spread by soil contact, then the biologist shall prescribe methodologies for reducing spread from mechanical methods of vegetation management. These methods would likely be similar to those identified in BMP-4 through BMP-7 including, but not be limited to, washing equipment after working in infected areas, and planning work to progress from uninfected areas to infected areas.				located and plan work accordingly to prevent spread **During Activity:** Implement measures to prevent spread, such as by cleaning vehicles between work locations, if needed **After Activity:** N/A	
Impact Biology-1 **MM Biology-5: Roosting Bats** ***Broadcast Burning*** Prior to conducting broadcast burning, a qualified biologist shall review the selected location to determine whether potential roosting bat habitat is present. If adequate roosting trees are present, one of two options may be pursued: (1) A qualified bat biologist shall first conduct a focused assessment of the roosting habitat within 2 days of burning to determine whether bats are present. If bats are present. the bat biologist shall determine whether the broadcast burn poses a threat to the roosting bats based on the location of the bats as compared with the prescribed burn location, wind directions, and type of fuel to be burned. If bats could be within direct line of smoke, a threat would occur If a threat could occur, the broadcast burn must be conducted when ambient temperatures are warmer to allow escape of the bats or the tree(s) avoided. (2) The broadcast burn will be conducted, avoiding the potential roosting trees. ***Tree Removal*** Prior to the removal of trees with a DBH of greater than 10", a qualified biologist shall conduct a focused tree habitat assessment. Trees containing suitable potential bat roost habitat features shall be clearly marked or identified. If day roosts are found to be potentially present, the biologist shall prepare a site-specific roosting bat protection plan to be implemented. Based on site-specific conditions, the plan should incorporate the following guidance as appropriate: **Roost Avoidance** When possible, removal of trees identified as providing suitable roosting habitat should be conducted during seasonal periods of bat activity, including: • Between March 1 and April 15, or after evening temperatures rise above 45 degrees Fahrenheit and/or no more than ½ inch of rainfall within 24 hours occurs; or • Between September 1 and about October 15, or before evening temperatures fall below 45 degrees Fahrenheit and/or more than ½ inch of rainfall within 24 hours occurs. If it is determined that a colonial maternity roost is potentially present, the roost shall be avoided and shall not be removed during the breeding season (April 15 to August 31) unless removal is necessary to address an imminent safety hazard. Operation of mechanical equipment producing high noise levels (e.g., chainsaws, heavy equipment) in proximity to buildings/structures supporting or potentially supporting a colonial bat roost shall be restricted to periods of seasonal bat activity (as defined above), when possible. **Assessment** If work with loud, mechanical equipment must occur near a known or potential roosting structure/building during the maternity or hibernation roosting periods, then a qualified bat	Contractor working with qualified biologist	The District	Where trees in bat roosting habitat could be impacted by activities (predominantly MA-21, MA-23, and MA-24)	**Before Activity:** (1) Conduct surveys if tree removal could occur in bat roosting areas and work is occurring during roosting, (2) humanely evict bats, if appropriate **During Activity:** Avoid roosting bats **After Activity:** N/A	

Best Management Practice and Mitigation Measure	Implementation Responsibility	Monitoring Responsibility	Applicable Locations	Timing and Performance Standards	Compliance Verification
biologist shall first conduct a focused assessment of the structure. The site-specific plan shall be implemented to prevent noise-related impacts on roosting bats. **Roost Removal** If a tree potentially containing a colonial maternity roost must be removed, such as in the event of unsafe conditions requiring treatment, during the breeding season, then the following or other measures recommended by the qualified bat biologist may be implemented: • Acoustic emergence surveys or other appropriate methods shall be conducted/implemented to further evaluate if the roost is an active maternity roost. • If it is determined that the roost is not an active maternity roost, then the roost may be removed in accordance with the other requirements of this measure; • If it is found that an active maternity roost of a colonial roosting species is present, the roost shall not be disturbed during the breeding season. Potential colonial hibernation roosts will only be removed during seasonal periods of bat activity (i.e., non-hibernation periods). Potential non-colonial roosts that cannot be avoided shall be removed on warm days in late morning to afternoon when any bats present are likely to be warm and able to fly. Appropriate methods shall be used to minimize the potential of harm to bats during tree removal. Such methods may include using a two-step tree removal process. This method is conducted over two consecutive days, and works by creating noise and vibration by cutting non-habitat branches and limbs from habitat trees using chainsaws only (no excavators or other heavy machinery) on Day 1. The noise and vibration disturbance, together with the visible alteration of the tree, is very effective in causing bats that emerge nightly to feed, to not return to the roost that night. The remainder of the tree is removed on Day 2.					
Impact Biology-1 **MM Biology-6: Protection of Badgers** Prior to prescribed (broadcast and pile) burning, or prior to use of heavy equipment to remove and/or masticate vegetation in badger denning habitat, which is characterized by herbaceous, shrub, and open stages of most habitats with dry, friable soils, a qualified wildlife biologist shall conduct a survey to identify any American badger burrows/dens. These surveys shall be conducted not more than 15 days prior to the start of work. American badger dens determined to be occupied during the breeding season (February 15 through June 30) shall be flagged, and ground-disturbing activities avoided within 100 feet to protect adults and nursing young. Buffers may be modified by the qualified biologist, provided the badgers are protected, and shall not be removed until the qualified biologist has determined that the den is no longer in use. If the den is occupied during the non-maternity period (July 1 through February 14) and avoidance is not feasible, a passive badger relocation plan will be prepared and submitted to the CDFW for approval. Any passive relocation of American badgers shall occur only under the direction of a qualified biologist and with CDFW approval.	Contractor working with qualified wildlife biologist	The District	Wherever broadcast burning or use of heavy equipment that could disturb ground (excluding mowers in fuelbreaks or defensible spaces) could be used in badger denning habitat	**Before Activity:** Conduct surveys, as needed **During Activity:** Maintain non-disturbance areas around active dens or evict, as appropriate **After Activity:** N/A	
Impact Biology-1 **MM Biology-7: Protection of Nesting Birds** If mowing with heavy equipment or other vegetation (including tree) removal activities or prescribed (broadcast and pile) burning would commence anytime during the nesting/breeding season of native bird species (February 1 to September 1), a pre-construction survey for nesting birds shall be conducted by a qualified biologist within seven days of the habitat disturbance. The survey shall include visually surveying all suitable nesting habitat in the survey area, and be conducted during periods of high bird activity (i.e., 1-3 hours after sunrise and 1-3 hours before sunset). When the activity would occur along an existing fuel break or in other areas that are currently maintained such as along roads and in defensible spaces, then the survey area shall include only the disturbance footprint. During the	Contractor working with qualified biologist	The District	Wherever heavy or noise equipment is used to implement BFFIP management actions	**Before Activity:** (1) Conduct surveys, if appropriate, (2) identify nest buffers as needed **During Activity:** Maintain non-disturbance areas around active nests. **After Activity:** N/A	

Best Management Practice and Mitigation Measure	Implementation Responsibility	Monitoring Responsibility	Applicable Locations	Timing and Performance Standards	Compliance Verification
construction of new fuelbreaks or during vegetation removal with heavy equipment in areas that were not previously managed (such as under MA-23 and MA-24), the survey area shall include the disturbance area and a surrounding buffer to be determined by a qualified biologist depending on type of equipment used, vegetation community, topography, resident bird species, and any other relevant factors. If active nests of bird species protected by the Migratory Bird Treaty Act and/or the California Fish and Game Code are found in areas that could be directly or indirectly disturbed (noise), a no-disturbance buffer zone shall be created around active nests during the breeding season or until a qualified biologist determines that all young have fledged. The size of the buffer zone shall be determined by the biologist, by taking into account factors including but not limited to the following: 1. Noise and human disturbance levels at the site at the time of the survey and the noise and disturbance expected during the vegetation management activity; 2. Distance and amount of vegetation or other screening between the site and the nest; and 3. Sensitivity of individual nesting species and behaviors of the nesting birds.					
Impact Biology-1 **MM Biology-8: Northern Spotted Owl Avoidance During Nesting Season** If mowing with heavy equipment, the mechanical removal of vegetation, or prescribed burning, including pile and broadcast burning, is to occur within the northern spotted owl nesting season (February 1 to July 31), the District shall commission two surveys for nesting northern spotted owls during the months of April and May preceding the commencement of these activities. At a minimum, the survey area shall include all suitable nesting habitats within 0.25 mile of any planned activity sites, and then one of the two options listed below shall be implemented: 1. Following a round of protocol-level northern spotted owl surveys in accordance with the USFWS *Protocol for Surveying Proposed Management Activities that may Impact Northern Spotted Owls* (USFWS, 2012), if it is conclusively determined that there are nesting northern spotted owls, planned activities that generate noise (e.g., mowing, heavy equipment usage) that are within 0.25-mile of an identified active nest shall not begin prior to September 1 unless the young have fledged, at which time work may begin no earlier than July 10. Prescribed burns may only occur within suitable northern spotted owl habitat (as determined by a qualified biologist) during the nesting season if protocol surveys have determined that northern spotted owl nesting is not occurring. 2. Alternatively, the District shall perform a calculation to determine the minimum buffer needed to avoid impacts on this species from noise generation by equipment. The calculation shall be based on the guidance and methodology in the USFWS "Transmittal of Guidance: Estimating the Effects of Auditory and Visual Disturbance to Northern Spotted Owls and Marbled Murrelets in Northwestern California," (USFWS, 2006) which takes into consideration the baseline noise levels, the noise and duration of noise generated by the loudest equipment, and the topography of the landscape. The resulting buffer calculated using these methods shall be a minimum buffer, but in no case shall the buffer be less than 500 feet. If the calculation is not performed, a conservative 0.25-mile buffer shall be implemented per (1), above. If nesting northern spotted owls are found, activities shall not occur prior to September 1 unless the young have fledged, at which time work may begin no earlier than July 10. Manual methods shall not occur within 131 feet of the line-of-site of a nesting northern spotted owl.	Contractor working with qualified biologist	The District	Any areas of the District's lands where northern spotted owls can occur, including the Watershed and the Nicasio administrative unit	**Before Activity:** (1) Conduct surveys, (2) as appropriate calculate buffer distances or conduct work outside of nesting season **During Activity:** Maintain buffers **After Activity:** N/A	

Best Management Practice and Mitigation Measure	Implementation Responsibility	Monitoring Responsibility	Applicable Locations	Timing and Performance Standards	Compliance Verification
Impact Biology-1 **MM Biology-9: Protection of Western Pond Turtle Nesting Habitat and Overwintering** **Nesting** Any mechanical method of vegetation management (i.e., heavy equipment), vehicle travel, or prescribed (broadcast and pile) burning that could occur where suitable western pond turtle nesting habitat is present shall be reviewed by a qualified biologist to determine if western pond turtle nesting could be present in the area. If the work with heavy equipment were to occur in loose soils in oak woodlands, mixed coniferous forests, broadleaf forests, or grasslands that are within 100 feet of ponds, during the western pond turtle egg-laying season (May to August) as determined by the qualified biologist, the activity shall either be rescheduled to occur outside of the egg-laying period; or a survey shall be conducted to determine if eggs and nests are present in the work area and any identified eggs or nests and young turtles shall be avoided. **Overwintering of Hatchlings in Nests** Any mechanical method of vegetation management (i.e., heavy equipment) or vehicle travel that could occur where suitable overwintering habitat for hatchlings is present shall be reviewed by a qualified biologist to determine if any hatchlings could be present in the area. If work with heavy equipment were to occur in loose soils in oak woodlands, mixed coniferous forests, broadleaf forests, or grasslands that is within 225 meters of ponds known to be used by the western pond turtle, during the overwintering season (October to April) (Holland, 1994) as determined by the qualified biologist, the activity shall either be rescheduled to occur outside of the overwintering period, or a survey shall be conducted to determine if hatchlings are present in the work area and any identified nests shall be avoided.	Contractor working with qualified biologist	The District	Wherever heavy equipment, vehicle travel, or prescribed burning could occur in western pond turtle breeding habitat during their breeding season (May to August) or where heavy equipment and vehicle travel could occur during the overwintering season for hatchlings (October to April)	**Before Activity:** (1) Biologist determines if the work area could support pond turtle breeding or overwintering based on the location of the work and proximity to ponds, (2) if no pond turtle could occur, work can proceed, (3) if pond turtle could be found in an area, the area shall be avoided or work rescheduled, (4) a survey can also be performed to rule out pond turtle eggs or overwintering hatchlings from the work area **During Activity:** Avoid pond turtle nests or overwintering hatchlings, if any had been found in surveys **After Activity:** N/A	
Impact Biology-1 **MM Biology-10: California Red-Legged Frog Avoidance** Prior to implementing any vegetation management activities involving vehicles or equipment (i.e., mowers, graders, skid steer loader) within 0.25 mile of Lagunitas Creek downstream of Kent Lake, or around Soulajule Reservoir (or any location where California red-legged frogs have been found), a qualified biologist shall conduct protocol-level in accordance with the USFWS *Revised Guidance on Site Assessments and Field Surveys for the California Red-legged Frog* (USFWS, 2015) surveys the areas where activities are to occur to ensure that no California red-legged frogs are present in the activity footprint. The biologist shall also mark the work area and the maintenance crew shall be directed to stay within the marked activity areas. If California red-legged frogs are found, no work shall occur until the frogs have moved on their own from the activity area.	Contractor working with qualified biologist	The District	Locations where California red-legged frog have been observed or within designated critical habitat	**Before Activity:** (1) Conduct a survey for any individuals in the work area, (2) if California red-legged frogs have been observed or if work is to occur within designated critical habitat, prior use of vehicles or equipment **During Activity:** If observed, activities must not occur until the individual(s) leave the area **After Activity:** N/A	
Impact Biology-1 **MM Biology-11: Marin Elfin Butterfly Host Plant Avoidance** Prior to vegetation management activities in the limited areas where stonecrop is known to occur (steep slopes on southeast shore of Lake Lagunitas, north-facing slopes south of Alpine Lake, and north of Kent Lake), District botanical staff shall be notified. If the activity would occur in an area containing or potentially containing stonecrop, then a survey shall be conducted to flag all stonecrop plants within and bordering the work area. Work crews shall be instructed to avoid flagged plants or larger areas, and work crews shall be trained in identification of stonecrop.	Contractor working with the District's botanical staff	The District	Locations where stonecrop is known to occur (steep slopes on southeast share of Lake Lagunitas, north-facing slopes south of Alpine Lake, and north of Kent Lake)	**Before Activity:** (1) Determine if activity could occur in the limited areas where stonecrop may also occur, (2) conduct survey for stonecrop if there is overlap. **During Activity:** Avoid stonecrop **After Activity:** N/A	
Impact Biology-1 **MM Biology-12: Protection of Foot-Hill Yellow Legged Frog** Immediately prior to the use of heavy equipment, any other ground disturbing Plan activities, or prescribed (broadcast and pile) burning within 50 feet of Big Carson Creek, Little Carson Creek, or their tributaries, a clearance survey for foothill yellow-legged frog shall be	Contractor working with trained individual and qualified biologist	The District	Activities (not including manual methods or planting) within 50 feet of Big Carson Creek, Little Carson Creek, or their tributaries	**Before Activity:** (1) Survey for the species **During Activity:** If observed, activities must not occur until the individual(s) leave the area	

Best Management Practice and Mitigation Measure	Implementation Responsibility	Monitoring Responsibility	Applicable Locations	Timing and Performance Standards	Compliance Verification
conducted by an individual trained in the identification of the species. If foothill yellow-legged frogs are found, no work shall occur until the frogs have moved on their own from the activity center.				**After Activity:** N/A	
Impact Biology-1 **MM Biology-13: Mollusk Avoidance** Only hand methods of removal shall be used when working directly in seeps or springs, unless a survey for Marin Hesperian and robust walker is undertaken. If the species are not found in surveys, the work can proceed. If individuals are found, the area should be avoided or work shall only proceed using hand methods, supervised by a qualified biologist. If the use of equipment other than hand tools are required in Potrero Meadow, then a site-specific protection plan for Marin Hesperian and robust walker shall be prepared by a qualified biologist. The plan may include conducting clearance surveys and having a qualified monitor onsite during construction activities, as well as ensuring that activities in that area would protect and/or enhance habitat in that area in the long-term.	Contractor working with qualified biologist	The District	The locations where treatments could need to occur in habitat suitable for Marin Hesperian and Robust Walker (i.e., springs or seeps)	**Before Activity:** Survey for the species if work could occur in their habitat **During Activity:** Avoid the species or only perform hand work in the immediate vicinity of the species **After Activity:** N/A	
Impact Biology-1 **MM Biology-14: Northern Spotted Owl** **Projects Within 0.25 Mile of an Activity Center** *Determine Type of Habitat Present* Prior to vegetation management within an area the latest GIS data available for northern spotted owl activity centers shall be consulted to determine whether the project is within 0.25 mile of an activity center. Once determined to be within 0.25 mile of an activity center, the habitat shall be reviewed to determine whether the project is proposed to occur within a forest habitat type that provides potential northern spotted owl foraging, roosting, and/or nesting habitat. This may be accomplished as follows: 1. A review of GIS data shall be conducted to determine if the activity is proposed to occur in a forest type potentially used by northern spotted owls (i.e., Douglas-fir, redwood, mixed conifer/hardwood forest, mature broadleaf/evergreen forest types). If the activity would not occur within a forest type potentially used by northern spotted owls, then no further actions is required to protect northern spotted owl habitat. 2. If the project is proposed to occur in a forest type potentially used by northern spotted owls, then a site-specific habitat evaluation shall be conducted within the month of February prior to the activity by a qualified northern spotted owl biologist to determine if the area provides the required habitat characteristics to provide northern spotted owl foraging, roosting, and/or nesting habitat. *Projects Within Appropriate Habitat* For projects which are proposed to occur in potential northern spotted owl foraging, roosting, or nesting habitat, the following action shall be implemented prior to management activities: 1. Habitat alteration within core use areas (nesting and roosting habitat) shall be planned and conducted under the guidance of a qualified northern spotted owl biologist. Opportunities to conduct vegetation management to enhance development of late- successional characteristics or to meet other restoration goals in a manner compatible with retaining resident northern spotted owls shall be evaluated and implemented. Restoration activities conducted near northern spotted owl sites shall first focus on areas of younger forest less likely to be used by northern spotted owls and less likely to develop late-successional forest characteristics without vegetation management. Vegetation management projects shall be designed to include a mix of disturbed and undisturbed areas, retention of woody debris, and development of understory structural diversity to maintain small mammal populations across the landscape.	Contractor working with qualified northern spotted owl biologist	The District	Areas within 0.25-mile of where northern spotted owls could forage, roost, or next	**Before Activity:** (1) Consult GIS layers to determine if a project would occur in northern spotted owl activity areas, (2) conduct surveys to evaluate habitat if work is to occur in a forest that could support northern spotted owls **During Activity:** Alter habitat as specified in measure, avoid woodrat stick nests **After Activity:** N/A	

Best Management Practice and Mitigation Measure	Implementation Responsibility	Monitoring Responsibility	Applicable Locations	Timing and Performance Standards	Compliance Verification
2. Presumed active woodrat stick nests (i.e., with visible signs of activity as determined by the qualified biologist) would be temporarily demarcated during surveys by the qualified biologist. Woodrat stick nests and areas around the nests, shall be avoided during vegetation management activities. Any flagging or other markings would be removed following the activity.					
Impact Biology-1 **MM Biology-17: Protection of California Giant Salamander** Immediately prior to the use of heavy equipment, any other ground disturbing Plan activities, or prescribed (pile and broadcast) burning within 50 feet of a stream or within riparian habitat, a clearance survey for California giant salamander shall be conducted by an individual trained in the identification of the species. Any identified California giant salamander shall be relocated (by a qualified biologist in possession of a valid Scientific Collecting Permit, or appropriate permit at the time of work if listing status changes) to a suitable nearby location at least 250 feet from the original loction. Alternatively, the activity may be delayed until the salamander has left the area on its own.	Contractor working with trained individual and qualified biologist	The District	Activities (not including manual methods or planting) within 50 feet of a stream or within riparian habitat	**Before Activity:** (1) Survey for the species, (2) move any individuals found in the work footprint prior to conducting activities **During Activity:** N/A **After Activity:** N/A	
Impact Biology-1: Implement Mitigation Measures MM Geology-1, MM Geology-3, and MM Hydrology-1 (see below)					
Impact Biology-2: Implement Mitigation Measures MM Biology-1, MM Biology-2, MM Biology-3, and MM Biology-4 (see above), MM Geology-1 and MM Geology-3 (see below), and Best Management Practices BMP-1 through BMP-7 (see above).					
Impact Biology-2 **MM Biology-15: Protection of Wetlands** All projects involving mowing with heavy equipment or mechanical removal with heavy equipment shall be evaluated by a qualified biologist prior to initiation of the work. If the biologist determines that the project would occur in an area where wetlands are known or potentially present, the following avoidance and minimization measures shall be implemented: • Prior to mowing or mechanical removal, all wetlands in the disturbance area shall be flagged (or otherwise demarcated) and heavy equipment shall not operate within the flagged area(s); or • Heavy equipment may be operated in a seasonal wetland only when the wetland is dry (as determined by the biologist); or • Only heavy equipment designed to operate within wet or saturated soils may be used. The equipment must be able to operate without causing rutting, compaction of soils, or other soil and topography disturbances. If rutting or soil compaction occurs, these areas shall be restored prior to the wet season.	Contractor working with qualified biologist	The District	Areas where wetlands could occur	**Before Activity:** (1) Biologist reviews work areas to determine if work could occur in a wetland, (2) if yes, areas of wetlands shall be flagged for avoidance prior to conducting work **During Activity:** Use only equipment designated for use in wet, saturated soils **After Activity:** Restore any rutting before the wet season	
Impact Biology-2 **MM Biology-16: Protection of Native Grasslands** All projects involving mowing with heavy equipment, mechanical removal with heavy equipment, or grazing shall be evaluated by the District's biologist prior to initiation of the work. For the purposes of this measure, a native grassland community is defined as an area with a relative cover or absolute cover of native grasses that meets the "Membership Rules" defined in a Manual of California Vegetation (Sawyer, Keeler-Wolf, & Evens, 2009), and that has a minimum stand size of 0.25-acre. If the biologist determines that the project would occur in an area where native grassland communities are known or potentially present, the following avoidance and minimization measures shall be implemented: • Prior to mowing or mechanical removal, all native grassland communities in the disturbance area shall be identified. The District biologist shall then evaluate if the proposed activity may be detrimental to the grassland area. At a minimum, MM Biology-3 shall be implemented to prevent the spread of invasive species. As needed, the District biologist may also require the following:	Contractor working with the District's biologist	The District	Areas where mowing, heavy equipment, or grazing could be used in sensitive grasslands	**Before Activity:** Biologist reviews work areas to determine if work could occur in a sensitive grassland, (2) if yes, areas sensitive communities shall be flagged for avoidance prior to conducting work **During Activity:** Avoid flagged areas and only enter the sensitive grasslands after grasses have gone to seed when soils are dry **After Activity:** Monitor the grassland areas following the disturbance for any changes in its size or composition	

Best Management Practice and Mitigation Measure	Implementation Responsibility	Monitoring Responsibility	Applicable Locations	Timing and Performance Standards	Compliance Verification
– Flagging the boundaries of the sensitive grassland area and heavy equipment shall not operate within the flagged area(s); or – Heavy equipment may be operated in the area only after the grasses have gone to seed and when soils are dry; or – Monitoring of the grassland area following the disturbance to ensure that the cover of native grasses has not been altered by the activity, and the implementation of restoration activities as needed.					
Impact Biology-3: Implement Mitigation Measure MM Biology-1 (see above), MM Geology-3 and MM Hydrology-1(see below)					
Impact Biology-4: Implement Mitigation Measures MM Biology-3, MM Biology-5, MM Biology-6, MM Biology-7, MM Biology-8, and MM Biology-9 (see above), MM Geology-1, MM Geology-3, and MM Hydrology-1 (see below)					
Impact Biology-Cumulative: Implement Mitigation Measures MM Biology-1, MM Biology-2, MM Biology-3, MM Biology-4, MM Biology-5, MM Biology-6, MM Biology-7, MM Biology-8, MM Biology-9, and MM Biology-10 (see above), MM Geology-1, MM Geology-3, and MM Hydrology-1 (see below)					
Cultural and Tribal Cultural Resources					
Impact Cultural Resources-1 **MM Cultural-1: Cultural Resources Training** All employees and contractors shall receive cultural resource training conducted by a qualified cultural resources specialist (e.g., an archaeologist or tribal monitor, if appropriate) prior to working on BFFIP projects. For tracking purposes, a list of individuals who have received training shall be maintained at the District headquarters. The training shall address appropriate work practices necessary to effectively implement the mitigation measures (MM Cultural-2, -3, and -4), for historical resources, archaeological resources, tribal cultural resources, and human remains. The training shall address the potential for exposing subsurface resources, recognizing basic signs of a potential resource, understanding required procedures if a potential resource is identified including reporting the resource to a qualified archaeologist or cultural resources specialist, and understanding all procedures required under Health and Safety Code § 7050.5 and PRC §§ 5097.94, 5097.98, and 5097.99 for the discovery of human remains.	Contractor working with qualified cultural resources specialist	The District	BFFIP Area	**Before Activity:** Train employees and contractors how to implement the mitigation measures (MM Cultural-2 through MM Cultural-4) **During Activity:** N/A **After Activity:** N/A	
Impact Cultural Resources-1 **MM Cultural-2: Known Cultural Resources and Pre-Activity Surveys** The District shall maintain a confidential GIS database of all survey areas and discovered historic and archaeological resources in the BFFIP area. In the event that a Native American tribe identifies a prehistoric trail alignment on District land, the alignment shall be added to the confidential GIS database. Prior to conducting any work associated with the BFFIP, the work areas shall be compared against the GIS data to determine if the area has been previously surveyed and if it has been surveyed, if any historic or archaeological resources are found in the work area. Any resources that have not been evaluated shall be assumed eligible for listing in the CRHR and assumed significant. If the GIS data shows that the areas where soil -disturbance below the surface through use of heavy equipment, or burning is proposed have not been previously surveyed, consultation with the Tribe shall occur. Notification with maps of the location of work shall be provided to a Native American tribe identified by the NAHC to be traditionally and culturally affiliated with the geographic area of the project site. A pre-activity cultural resources survey shall be conducted by a qualified archaeologist or cultural resources specialist in accordance with industry standards prior to performing work, unless vegetation is too dense making a survey impossible. In the event vegetation is too dense, making a pre-activity survey challenging or impossible, the training conducted under MM Cultural-1, shall be sufficient to permit work to be conducted using only manual techniques accessed on foot. If historical or archaeological resources are located in the work area (either as identified in previous surveys or during pre-activity surveys), the resource, plus a 50-foot buffer, shall be	Contractor working with qualified archaeologist; the District	The District	BFFIP Area	**Before Activity:** Consult the GIS cultural resources layer for the presence of recorded sites **During Activity:** (1) Avoid recorded resources or impacts on resources or use only hand methods in resource areas, (2) Examine area where piles are proposed for resources **After Activity:** Remove resource delineators	

Best Management Practice and Mitigation Measure	Implementation Responsibility	Monitoring Responsibility	Applicable Locations	Timing and Performance Standards	Compliance Verification
avoided. For resources that are not readily evident in the field, the boundaries around the resource shall be temporarily marked such as with fencing or flagging. If work must commence in the sensitive area, it can only be performed using hand tools or powered hand tools, cannot include ground disturbance below the topsoil layer, and can only be accessed on foot. Alternatively, the resource can be evaluated for eligibility for the CRHR and reviewed by a tribal monitor to determine whether it constitutes a tribal cultural resource, if the resource is archaeological. If found ineligible and not a tribal cultural resource, work could proceed as normal. If found eligible or to be a tribal cultural resource, impacts on the resource must be avoided (through total avoidance of the area, or through use of hand methods only in the area of the resource, as described here). After work is completed, all cultural resource delineators (flags, fencing) shall be removed in order to avoid potential vandalism, unauthorized excavation(s), etc. Prior to stashing slash for pile burning, the areas where piles are proposed for location shall be examined by the workers creating the piles to ensure that no resources are located on the ground surface under the piles. All workers shall be trained in the identification of cultural resources. If a potential resource is identified, piles for burning shall be moved to avoid the resource(s) and MM Cultural-3 implemented.					
Impact Cultural Resources-1 **MM Cultural-3: Previously Unidentified Cultural Resources** In the event that a previously unidentified cultural resource is discovered during implementation of an activity all work within 165 feet (50 meters) of the discovery shall be halted. The resource shall be located, identified, and recorded in the District's cultural resources GIS identified in MM Cultural-2. Data regarding archaeological resources shall be shared with Native American tribes identified by the NAHC to be traditionally and culturally affiliated with the geographic area of the project site. A qualified cultural resource specialist/archaeologist shall inspect the discovery and determine whether further investigation is required. If the discovery can be avoided and no further impacts shall occur, the resource shall be documented on California State Department of Parks and Recreation cultural resource record forms and no further effort shall be required. If work must commence in the sensitive area, it can only be performed using hand tools or powered hand tools, cannot include ground disturbance below the topsoil layer, and can only be accessed on foot. Alternatively, the cultural resource specialist/ archaeologist shall evaluate the resource and determine whether it is: • Eligible for the CRHR (and a historical resource for purposes of CEQA), • A unique archaeological resource as defined by CEQA, and/or • A potential tribal cultural resource (all archaeological resources could be a tribal cultural resource). If the cultural resources specialist/archaeologist determines that the resource could be a tribal cultural resource, he or she shall, within 48 hours of the discovery, notify each Native American tribe identified by the NAHC to be traditionally and culturally affiliated with the geographic area of the project site of the discovery. A tribal monitor shall inspect the resource to determine whether it constitutes a tribal cultural resource. If the resource is determined to be neither a unique archaeological, an historical resource, or a potential tribal cultural resource, work may commence in the area. If the resource meets the criteria for either a historical resource, unique archaeological resource, and/or tribal cultural resource, work shall remain halted and the cultural resources specialist/archaeologist shall consult with the District staff regarding methods to ensure that no substantial adverse change would occur to the significance of the resource pursuant to CEQA Guidelines Section 15064.5(b). The responding tribes shall be given an opportunity to participate in determining the appropriate mitigation methods for tribal cultural resources in consultation with the District.	Contractor working with qualified archaeologist	The District	BFFIP Area	**Before Activity:** N/A **During Activity:** (1) Cease activity if a cultural resource is uncovered, (2) Avoid resource if possible, (3) Evaluate and determine whether the resource is eligible, unique, or could be a tribal cultural resource, (4) If the resource could be a tribal cultural resource, notify Native American tribe identified by the NAHC to be traditionally and culturally affiliated with the geographic area of the project site, (5) If the resource is not eligible, unique, and/or a tribal cultural resource, work may commence, (6) If the resource is eligible, unique, and/or a tribal cultural resource, work remains halted and a method selected to ensure that adverse change to the resource does not occur, (7) Preserve in place if possible, (8) If not possible to preserve in place, and as deemed appropriate by the qualified cultural resource specialist/archaeologist and tribal monitor, for tribal cultural resources, recover and record cultural materials. Once recovered and recorded, the activity can commence in this area. **After Activity:** Ensure resource has been appropriately recorded in District's cultural resources GIS.	

Best Management Practice and Mitigation Measure	Implementation Responsibility	Monitoring Responsibility	Applicable Locations	Timing and Performance Standards	Compliance Verification
Avoidance of the area, or avoidance of impacts on the resource, is the preferred method of mitigation for impacts on cultural resources and shall be required unless there are other equally effective methods. Other methods to be considered shall include evaluation, collection, recordation, and analysis of any significant cultural materials in accordance with a Cultural Resources Management Plan prepared by the qualified cultural resource specialist/archaeologist. The methods and results of evaluation or data recovery work at an archaeological find shall be documented in a professional level technical report to be filed with California Historical Resources Information System (CHRIS). Work may commence upon completion of evaluation, collection, recordation, and analysis, as approved by the qualified archeologist and tribal monitor, for tribal cultural resources.					
Impact Cultural Resources-2 **MM Cultural-4: Human Remains** The treatment of human remains and of associated or unassociated funerary objects discovered during any soil-disturbing activity within the proposed plan area shall comply with applicable State laws. • If human remains are at any time noted during activities around MRN-496/P-21-000445 or in the plan area, work shall be halted within 165 feet (50 meters) of the discovery. The professional archaeologist and the District shall notify the Marin County Coroner's office as prescribed in Public Resources Code §5097.98 and Health and Safety Code §7050.5. • In the event of the coroner's determination that the human remains are Native American, notification of the Native American Heritage Commission is required, who shall appoint a Most Likely Descendant (MLD) (PRC §5097.98). • The human remains shall be protected until a decision is reached on the final disposition of the remains. • The District, the professional archaeologist, and the MLD shall make all reasonable efforts to develop an agreement for the treatment, with appropriate dignity, of human remains and associated or unassociated funerary objects (CEQA Guidelines Section 15064.5[d]). The agreement should take into consideration the appropriate excavation, removal, recordation, analysis, custodianship, curation, and final disposition of the human remains and associated or unassociated funerary objects. If the MLD and the other parties do not agree on the disposition of the remains, the reburial method shall follow PRC §5097.98(b) which states that: . . . the landowner or his or her authorized representative shall reinter the human remains and items associated with Native American burials with appropriate dignity on the property in a location not subject to further subsurface disturbance.	Contractor, coroner, the District, the professional archaeologist, the MLD	Marin Municipal Water District	BFFIP Area	**Before Activity:** N/A **During Activity:** (1) Avoid known location of human remains, (2) Cease activity if human remains are uncovered, (3) Appoint a Most Likely Descendent, (4) Protect human remains until a decision is reached, (5) If avoidance is not possible, the District, professional archaeologist, and MLD, remove human remains and associated or unassociated funerary objects from the location and move to selected location in accordance to decision reached. Once moved then the activity can commence again in this area. **After Activity:** N/A	
Impact Cultural Resources-3: Implement Mitigation Measures MM Cultural-1, MM Cultural-2, MM Cultural-3, and MM Cultural-4 (see above)					
Impact Cultural Resources-Cumulative: Implement Mitigation Measures MM Cultural-1, MM Cultural-2, MM Cultural-3, and MM Cultural-4 (see above)					
Geology and Soils					
Impact Geology and Soils-1 **MM Geology-1: Erosion Control and Slope Stability Measures** Best management practices (BMPs) for forestry shall be implemented to ensure vegetation management does not result in erosion, loss of topsoil, or slope instability in areas where work could result in the exposure of bare soils or the loss of root-soil matrix strength. If groundcover is determined to be less than 70 percent[a] following work, then BMPs, as identified here, shall be implemented. Prior to conducting work in any given area under any management action that could result in erosion or slope instability (e.g., broadcast burns, tree removal, weed removal, or forest treatments that could reduce the groundcover and expose soil) the area shall be inspected	Contractor	The District	Any areas where the ground is disturbed and soils are exposed through vegetation management actions	**Before Activity:** Inspect areas for treatment prior to treatment to assess the potential for erosion and soil instability **During Activity:** Implement the protection measures as needed to avoid or minimize erosion and slope instability **After Activity:** Conduct inspections as needed after actions, depending on the size and nature	

Best Management Practice and Mitigation Measure	Implementation Responsibility	Monitoring Responsibility	Applicable Locations	Timing and Performance Standards	Compliance Verification
for existing signs of erosion or slope instability (e.g. rills, slumped soil). Depending on the slope and the downslope resources (roads that could be impacted if a slope failed, waterbodies or habitat that could be impacted from erosion, important habitat, etc.), erosion and slope stabilization measures shall be determined prior to implementation of work, based on the list below. Generally, if an action would expose soils (groundcover less than 70 percent), then measures to protect soils, minimize erosion, and prevent slope instability shall be implemented. The measures to be implemented shall depend on the site's specific characteristics and the type and extent of vegetation management work to be performed. The inspection and determination of appropriate measures shall be made by personnel with knowledge and experience in the application of erosion and slope stabilization BMPs through training or field experience with BMP installation. The personnel shall memorialize in writing their field observations, and corresponding recommendations regarding installation of BMPs. The following measures shall be implemented during work, if the activity would reduce groundcover by 70 percent or more and as applicable: • Minimize areas to be disturbed to the greatest extent feasible • Avoid use of heavy equipment on slopes greater than 30 percent • Shut down use of heavy equipment, skidding, and truck traffic when soils become saturated and unable to support the machines • Sow native grasses and other herbs on denuded areas where natural colonization or other replanting shall not occur rapidly; use slash or chips to prevent erosion on such areas • Use surface mounds, depressions, logs, rocks, trees and stumps, slash and brush, the litter layer, and native herbaceous vegetation downslope of denuded areas to reduce sedimentation and erosion, as necessary to prevent erosion or slope destabilization • Stabilize steep slopes (i.e., greater than 30 percent) with mats or natural materials after tree removal or weed removal and prior to planting, where soils are exposed and could erode • Broadcast burns shall be performed outside of perennial and intermittent streams, and riparian forest/woodland. A 50-foot buffer around perennial and intermittent streams shall be maintained when the broadcast burn is proposed on a slope greater than 30 percent and upslope of the stream. • Install approved erosion control measures and non-filament-based geotextiles when: – conducting substantial ground disturbing work (i.e., use of heavy equipment, pulling large vegetation) within 100 feet[b] and upslope of currently flowing or wet wetlands, streams, lakes and riparian areas; – causing soil disturbance on moderate to steep (10 percent slope and greater) slopes; and – following the removal of invasive plants from stream banks to prevent sediment movement into watercourses and to protect bank stability • Sediment control devices, if installed, shall be certified weed-free, as appropriate. Sediment control devices shall be inspected daily to ensure that they are in good repair and working as needed to prevent sediment transport into the waterbodies (and repaired as needed) • Prior to conducting ground disturbing work the weather forecast shall be consulted; No substantial ground disturbing work (i.e., use of heavy equipment, pulling large vegetation) shall occur during rain events and 48 hours after a rain event, defined as 0.5 inch of rain or greater within a 48-hour period, or until soils are determined to not saturated Once work is completed the areas shall be inspected as needed and as accessible but at least annually until groundcover exceeds 70 percent and it is clear that significant erosion and slope instability are not occurring. At that time, erosion control and slope stability devices shall be removed.				of the work and the site, to ensure that erosion is not occurring and to remove any erosion control devices once they are no longer needed	
Impact Geology and Soils-1	Contractor	The District	Broadcast burn areas	**Before Activity:** Determine fire lines	

Best Management Practice and Mitigation Measure	Implementation Responsibility	Monitoring Responsibility	Applicable Locations	Timing and Performance Standards	Compliance Verification
MM Geology-2: Fire Lines During Broadcast Burns One or more of the following measures shall be implemented during broadcast burns to reduce erosion from fire lines: • Use existing barriers such as roads, trails, or wet lines as fire lines • Restore fire lines upon completion of the burn if they would not be used again (unless they are existing roads, trails, or other permanent elements). Utilize erosion control measures, such as sediment traps, during restoration to reduce sedimentation impacts. Restoration shall occur prior to one month after the fire line was created, assuming the fire line will not be used by another burn in the same year • Design broadcast burn boundaries to avoid gullies and highly erodible soils to the fullest extent possible				**During Activity:** Set up provisions as specified in the measure **After Activity:** Restore fire lines upon completion of work	
Impact Geology and Soils-1 **MM Geology-3: Grazing Land and Trail Control** Methods shall be implemented to reduce the possibility that grazing trails form include the following: • Prohibit grazing within 100 feet of lakes/reservoirs, creeks, streams, riparian corridors, and wetlands. Install fencing 100 feet from streams and riparian areas to exclude livestock • Implement methods, which could include rotating or providing multiple feeding areas, to minimize congregation of animals in any one location • Limit the number of animals spent grazing in a particular sized area, using the stocking rate equation taking into account days assumed to graze, slope, yield of the land, number of animals, weight of animals, and other appropriate factors • Conduct surveys of the grazing area during active grazing, identify if trails or other erosion features are forming • Ensure there are appropriate rest periods between grazing in any one area to allow regrowth of plants • If grazing trails or damaged areas form, the bare area shall be remediated by decompacting the soil and discontinuing grazing in the area until the trails are revegetated • Install off-stream watering tanks • Install fencing to exclude livestock from grazing on steep slopes (generally slopes with more than 30 percent grade), unless accounted for in stocking rate equation • During surveys of active grazing, conduct ongoing surveillance of installed erosion control features around riparian areas and fences around riparian areas • Repair damaged fencing or erosion control features as necessary	Contractor	Marin Municipal Water District	Grazing areas	**Before Activity:** Install fencing as needed **During Activity:** (1) Limit number of animals in an area based on appropriate calculations and minimize congregation of animals in any one location, (2) Repair damaged fencing or erosion control features, and (3) Conduct surveys during grazing to identify problem areas **After Activity:** (1) Permit appropriate rest periods after grazing, and (2) Remediate any bare areas	
Impact Geology and Soils-2: Implement Mitigation Measures MM Geology-1, MM Geology-2, and MM Geology-3 (see above)					
Impact Geology and Soils-Cumulative: Implement Mitigation Measures MM Geology-1, MM Geology-2, and MM Geology-3 (see above)					
Greenhouse Gas Emissions					
Impact GHG-1: Implement Mitigation Measure MM Air-1					
Impact GHG-2: Implement Mitigation Measure MM Air-1					
Impact GHG-Cumulative: Implement Mitigation Measure MM Air-1					
Hazardous Materials and Fire Hazards					
Impact Hazards-1 **MM Hazards-1: Spill Prevention and Response**	Contractor and the District	The District	BFFIP Area	**Before Activity:** N/A **During Activity:** (1) Implement appropriate best management	

Best Management Practice and Mitigation Measure	Implementation Responsibility	Monitoring Responsibility	Applicable Locations	Timing and Performance Standards	Compliance Verification
The District shall, at a minimum, implement best management practices that address the following procedures related to the use of hazardous materials during construction: • Proper disposal or management of contaminated soils and materials (i.e., clean up materials) • Daily inspection of vehicles and equipment for leaks and spill containment procedures • Emergency response and reporting procedures to address hazardous material releases • Emergency spill supplies and equipment shall be available to respond in a timely manner if an incident should occur • Response materials such as oil-absorbent material, tarps, and storage drums shall be available in the plan area at all times during management activities and shall be used as needed to contain and control any minor releases • The absorbent material shall be removed promptly and disposed of properly • Use of secondary containment and spill rags when fueling • Discourage "topping-off" fuel tanks • All workers shall be trained on the specific procedures for hazardous materials and emergency response as an element of the required worker environmental training prior to working in the plan area				practices that limit the potential for spills, (2) Cleanup any inadvertent spills appropriately **After Activity:** N/A	
Impact Hazards-2 **MM Hazards-2: Avoidance of MVAFS Hazards** Workers shall avoid all existing and former buildings and facilities within MVAFS or until the site is found to not have contamination in excess of background levels.	Contractor	The District	Projects within MVAFS	**Before Activity:** N/A **During Activity:** Avoid existing and former buildings and facilities when conducting weed removal activities **After Activity:** N/A	
Impact Hazards-4: Implement Mitigation Measures MM Hazards-1 (see above) and MM Hazards-3 (see below) **MM Hazards-3: Fire Risk Reduction for Stockpiling and Pile Burning** Piles shall not be burned during the fire season. Pile burning shall only be allowed on days when fire is less likely to spread (e.g., wind speeds are less than 15 mph). All requirements of the BAAQMD shall be met, including any permit, notification, and reporting requirements. Public notification shall be provided at least 24 hours in advance of a burn to individuals within 1 mile and at trailheads and fire roads leading to the area with piles proposed for burning. The public notification shall include current contact numbers to the appropriate burn coordinator.	Contractor	The District	Wherever stockpiles of slash are made and piles burned	**Before Activity:** Notify public and obtain all permits and make all necessary notifications as required by BAAQMD and MCFD **During Activity:** (1) Ensure that piles are away from highly ignitable areas (2) Ensure proper weather conditions during pile burning (3) Ensure proper fire-fighting equipment is on-hand during pile burning **After Activity:** N/A	
Impact Hazards-4 **MM Hazards-4: Prescribed Burn Plan** Prescribed Burn Plans shall be prepared for each broadcast burn project or for a larger area covering several planned projects. The Prescribed Burn Plan shall include the following information, at a minimum: • Project purpose and predicted outcome • Project location • Fuel conditions (discussion of types of plants and trees within and adjacent to project area)	Contractor	The District	Broadcast burn projects	**Before Activity:** (1) Prepare Prescribed Burn Plan including all identified details, (2) Notify the public at least 24 hours prior to broadcast burn and obtain necessary permits form or provide necessary notifications to MCFD and BAAQMD, (3) Arrange for appropriate crew and equipment to be on-site **During Activity:** Implement Prescribed Burn Plan	

Best Management Practice and Mitigation Measure	Implementation Responsibility	Monitoring Responsibility	Applicable Locations	Timing and Performance Standards	Compliance Verification
• Allowable atmospheric conditions and times to conduct the burn for safety and smoke dispersal (i.e., wind speeds, temperature, humidity, moisture of vegetation). Prescribed Burn Plans shall specify that burns generally occur: – After the morning inversion layer and before the evening inversion layer – When the atmosphere is neutral to unstable – During the day, to avoid nighttime inversion layers – When wind speeds are high enough that the air is not stagnant (i.e., 5 mph) and low enough that the broadcast burn can be managed safely • Avoidance of high fire danger days (e.g., Red Flag Days and Fire Weather Watch) Have fire suppression crews on-site from the start of the fire season determined by CAL FIRE (usually mid-May to early June) to the end of fire season (mid-November) during broadcast and pile burns • The broadcast burn specialist shall determine an appropriate buffer between flammable infrastructure or buildings and the broadcast burn, which is dependent upon the types of vegetation burned, moisture, weather, and topography • Event day logistics (numbers and types of personnel and equipment required, personal protective equipment) • Contingency plans (i.e., location and response time of emergency response, secondary fire lines) • Public notification at least 24 hours in advance of the burn to individuals within 1.5 miles and at trailheads and fire roads leading to the area proposed for burning. The public notification shall include current contact numbers to the appropriate burn coordinator. • Agency notification and coordination as required • Requirements of BAAQMD and MCFD				**After Activity:** N/A	
Impact Hazards-4 **MM Hazards-5: Roads and Trails Around Broadcast Burns** **Trails and District-Use-Only Roads** District-use-only roads and trails shall be closed to public recreational access within at least 500 feet of the outermost edges of a broadcast burns. District-use-only roads and trails shall be posted and blockaded with temporary fencing or the like. Notices of closures shall be posted at the trail heads and on the District's website. Additional measures such as staffing trail head closures can be implemented as needed. **Public Roads** If possible, public roads within 500 feet of the outermost edges of a broadcast burn shall be closed in coordination with the appropriate agency (e.g., Caltrans, Marin County). In the event this is not feasible, due to volume of traffic or lack of alternative routes, a Traffic Control Plan shall be prepared and adopted, in coordination with the appropriate agency. The Traffic Control Plan shall include the following at a minimum: • Requirement to coordinate with local law enforcement (e.g., County Sheriff, California Highway Patrol) • Installation of temporary signage at intervals ahead of and adjacent to the broadcast burn indicating that a broadcast burn is in progress • Use of flaggers to slow traffic during the burn or stop traffic if wind conditions shift, resulting in smoke crossing the road	Contractor	The District	Within 500 feet of the outer edges of a broadcast burn	**Before Activity:** (1) Post notices of closures at trailheads and online, (2) Prepare Traffic Control Plan **During Activity:** (1) Place blockades along District-use-only roads and trails, (2) staff closures of District-use-only roads and trails, if needed, (3) Implement Traffic Control Plan for public roads adjacent to broadcast burns **After Activity:** Remove blockades and signage	
Impact Hazards-4 **MM Hazards-6: Propane Flaming Training** Workers shall be trained prior to use of a propane torch. The training shall specify that, at a minimum, areas treated with a propane torch shall be monitored until it is clear that no smoke, smoldering vegetation, or flames are present.	Contractor	The District	In areas treated with a propane torch	**Before Activity:** Train workers for safe use of a propane torch **During Activity:** Monitor areas where propane flaming has been	

Best Management Practice and Mitigation Measure	Implementation Responsibility	Monitoring Responsibility	Applicable Locations	Timing and Performance Standards	Compliance Verification
				used for potential fires prior to leaving **After Activity:** N/A	
Impact Hazards-4: Implement Mitigation Measures MM Air-4 (see above) and MM Hazards-7 (see below) **MM Hazards-7: Fire Ignition and Spread Reduction** The following provisions shall be implemented during all management actions that involve the use of equipment that can generate sparks or heat: • Maintain fire suppression equipment in work vehicles • Closely monitor for ignited vegetation from equipment and tool use • Observe Red Flag Day and Fire Weather Watch warnings • Train workers to properly handle and store flammable materials, minimize potential ignition sources • Prohibit smoking in any vegetated areas	Contractor	The District	BFFIP Area	**Before Activity:** N/A **During Activity:** Ensure that measures are being implemented **After Activity:** N/A	
Impact Hazards-5: Implement Mitigation Measures MM Hazards-1, MM Hazards-3, MM Hazards-4, MM Hazards-5, MM Hazards-6, MM Hazards-7, and MM Air-4 (see above)					
Impact Hazards-6: Refer to individual analyses of MA-20 and MA-21 for application of mitigation measures pertinent to installation of fuelbreaks.					
Impact Hazards-7: Implement Mitigation Measures MM Geology-1 and MM Geology-2 (see above)					
Impact Hazards-Cumulative: Implement Mitigation Measures MM Geology-1, MM Geology-2, MM Hazards-3, and MM Air-4 (see above)					
Hydrology and Water Quality					
Impact Hydrolgoy-1 **MM Hydrology-1: Water Quality Protection During Waterway Crossing or Work Near Waterbodies** Vehicles and heavy equipment shall avoid instream crossings. If instream (waterway) crossings must occur because no other options for access are reasonably available, the crossing shall be performed when the stream is dry and soils are not saturated. The crossing shall be performed in a way that does not result in any permanent alteration of the stream bank or bed (e.g., choosing areas with stable soils and the least slope or with vegetation to protect the bed and bank). If water is flowing or the stream has flow or saturation, temporary plates or the equivalent shall be installed from bank to bank so for equipment to access across the waterway. If an instream crossing that could impact the bank or bed or riparian vegetation is needed, the crossing shall only be performed after and in accordance with the appropriate 1600 Streambed Alteration permit from CDFW and Section 404 and 401 Clean Water Act permits. All soils shall be restored after the instream crossing and banks revegetated after the work is completed, in accordance with permits.	Contractor	The District	Anywhere vehicles and heavy equipment must cross streams or creeks	**Before Activity:** (1) Obtain permits, (2) install plates or record vegetative conditions, as appropriate **During Activity:** Minimize soil or vegetation disturbance, as appropriate **After Activity:** Restore crossing area	
Impact Hydrolgoy-1: Implement Mitigation Measures MM Geology-1, MM Geology-2, MM Geology-3, and MM Hazards-1 (see above)					
Impact Hydrolgoy-3: Implement Mitigation Measures MM Hydrology-1, MM Geology-1, MM Geology-2, MM Geology-3, and MM Hazards-1 (see above)					
Impact Hydrology-Cumulative: Implement Mitigation Measures MM Hydrology-1, MM Geology-1, MM Geology-2, MM Geology-3, and MM Hazards-1 (see above)					
Noise					
Impact Noise-1: Implement Mitigation Measures MM Air-3 and MM Hazards-5 (see above), and MM Noise-1 (see below) **MM Noise-1: Noise Reduction Measures** **Work Timeframe Restrictions Near Sensitive Receptors**	Contractor and the District	The District	BFFIP Area	**Before Activity:** (1) Notify affected parties 1 week before, if applicable; (2) Conduct noise study, if desired	

Best Management Practice and Mitigation Measure	Implementation Responsibility	Monitoring Responsibility	Applicable Locations	Timing and Performance Standards	Compliance Verification
Work within 180 feet of a sensitive receptor shall only occur Monday through Friday from 7 am to 6 pm and Saturdays from 9 am to 5 pm, with no work allowed on Sundays or holidays, to follow the requirements of the Marin Countywide Plan (NO-1.i).				**During Activity:** (1) A designated coordinator shall ensure setbacks or other conditions are implemented: (2) Maintain buffer between receptor and equipment, if needed **After Activity:** N/A	

Near Residences and Ranger Residences

For activities that occurs in any one location (1,000 square foot area) for longer than 5 days within a 30-day period, the following noise buffers for equipment shall be implemented:

Equipment	Buffer Between Equipment and Sensitive Receptors (feet)
Backhoe/ Brushcutter	80
Chainsaw/ Excavator	113
Chipper	180
Generator/ Water pump	127
Fire engine	71
Leaf blower	64
Skid steer	90

- If these restrictions are not implementable between residences and a given location, the District shall notify the resident or contact at the sensitive receptor within 1 week of conducting the work. Work shall be coordinated to minimize disturbance to the receptor, such as conducting the work when no one is there. Noise barriers or other means could also be used, if necessary, to keep noise levels below 70 dBA. The District shall designate a disturbance coordinator to address any noise complaints under these circumstances.
- If these restrictions are not implementable between ranger residences and a given location, the District shall coordinate work with rangers at ranger residences to conduct work lasting more than 5 days within a 30-day period, to a time when rangers are not in the residences or when they would not be disturbed by the noise.

Near Cushing Memorial Amphitheater

- Coordinate with operators at Cushing Memorial Amphitheater to conduct work outside of event times.

Near Schools

- Coordinate work with Deer Park School and the San Anselmo Children's Center to occur when classes or other instructional activities are not occurring for any work involving mechanical/powered equipment that would last longer than 1 day and could cause noise to exceed 70 dBA at the school or childcare center.

Noise Study

If the District, based on their extensive history of conducting vegetation management activities, questions whether a noise level of 70 dBA may actually be exceeded by equipment at a sensitive receptor per the analysis in this section, the District may undertake a noise study to measure actual noise levels from equipment used during management actions to recalibrate the distances listed here. The noise study would be conducted by a noise consultant to industry standards. Resultant noise levels at sensitive receptors cannot exceed 70 dBA if the work lasts for more than 10 days near residences, ranger residences, and Cushing Memorial Amphitheater, or for more than 1 day near a school.

Impact Noise-Cumulative: Implement Mitigation Measure MM Noise-1 (see above)

Best Management Practice and Mitigation Measure	Implementation Responsibility	Monitoring Responsibility	Applicable Locations	Timing and Performance Standards	Compliance Verification
Recreation					
Impact Recreation-1: Implement Mitigation Measures MM Hazards-5 (see above) and MM Recreation-1 (see below) **MM Recreation-1: Protection of Recreationalists Along Trails and Roads** The following measures shall be implemented when management actions require heavy equipment or generate other hazardous conditions along roads and trails: • Close roads or trails when they are being used regularly by heavy trucks, transporting heavy equipment, or other large equipment that poses a hazard to recreationalists • Provide a road guard to usher recreationalists around hazards where work could impede on a road or trail, such as for stockpiling removed trees or vegetation. • Provide fencing to protect recreationalists from active work, as necessary. • Provide signage at trailheads at least one week prior to closure indicating that work may be occurring along the trails and for recreationalists to use caution.	Contractor	The District	Anywhere that implementation of management actions could pose a hazard to recreationalists	**Before Activity:** Post notices at least one week prior to trail closure **During Activity:** Use road guards, fences, or implement closures as appropriate as work is being conducted **After Activity:** Remove signage, as appropriate	
Impact Recreation-Cumulative: Implement Mitigation Measure MM Recreation-1 (see above)					
Transportation					
Impact Transportation-2: Implement Mitigation Measures MM Recreation-1 and MM Hazards-5 (see above)					
Impact Transportation-3 **MM Transportation-1: Emergency Access** The District shall ensure emergency access to the plan area along public roads is maintained during work. The following measures shall be implemented to ensure access is maintained: 1. In the event of an emergency, roads blocked or obstructed for maintenance activities shall be cleared to allow the vehicles to pass. 2. The District shall use road guards equipped with two-way radios during temporary lane or road closures. During an emergency, road guards will radio to the crew to cease operations and reopen the road to emergency vehicles. 3. All District authorized vehicles at the treatment site shall be parked so they do not block roads when there is no operator present to move the vehicle. The District shall contact the fire district or other emergency response agency with jurisdiction over the road subject to temporary closure to ensure that the agency is notified of the closure in advance.	Contractor and the District	The District	All locations on district lands where roads or trails may be blocked to perform work	**Before Activity:** N/A **During Activity:** Inform emergency responders of road closures and ensure road guards, and crew are equipped with two-way radios **After Activity:** N/A	
Impact Transportation-Cumulative: Implement Mitigation Measure MM Transportation-1 (see above)					

This page is intentionally left blank.

APPENDIX A:
PROJECT SPECIFIC REVIEW

A. PROJECT-SPECIFIC REVIEW

A.1 INTRODUCTION

The Biodiversity, Fire, and Fuels Integrated Plan (BFFIP) directs implementation of vegetation management actions in the Marin Municipal Water District's (MMWD's, or the District's) lands within Mount Tamalpais Watershed, and the shorelines of Nicasio and Soulajule Reservoirs to reduce wildfire risks and to preserve and enhance existing biological resources. The Program Environmental Impact Report (EIR) evaluated the environmental impacts of the BFFIP. The BFFIP is described in Chapter 2: Project Description of the Program EIR and within the BFFIP that is incorporated into the Program EIR by reference. The Program EIR was prepared under the direction of the CEQA Lead Agency, MMWD, in accordance with the California Environmental Quality Act (CEQA) (Public Resources Code [PRC] Section 21000 et seq.), and as a Program EIR in accordance with CEQA Guidelines Section 15168 for streamlining of CEQA review of later activities consistent with the BFFIP Program EIR.

The District will implement vegetation management actions consistent with the BFFIP. The District will prepare Annual Work Plans identifying the vegetation management actions proposed for each upcoming year. In accordance with the CEQA Guidelines, the lead agency must document evaluation of later activities to determine whether the environment effects of the activities are within the scope of the Program EIR (Section 15168(4)). The vegetation management activities proposed by the District each year constitute "later activities" in the context of the CEQA Guidelines. This document functions to aid the District in determining whether the later activities proposed by the District are within the scope of the BFFIP analyzed in the Program EIR or if additional environmental review is required. This document also serves to assist in the identification of Best Management Practices (BMPs) and Mitigation Measures (MMs) applicable to BFFIP actions taken, as required under the Program EIR.

A.2 DETERMINING WHETHER ACTIONS ARE WITHIN THE SCOPE OF THE BFFIP PROGRAM EIR

The purpose of the project-specific review is to determine and document whether the actions to be taken by MMWD are within the scope of the Program EIR. The BFFIP is comprised of Management Actions (MA). The MAs include various strategies, identify methods to implement the work, identify the zones where the work could occur, and identify the maximum acres of treatments that can be performed per year. The following table provides a summary of each of these components of the BFFIP, which were analyzed and included in the BFFIP Program EIR. Note that MA-1 through MA-18 include administrative and survey and monitoring actions that would not have impacts on the environment and are not included in the table.

A PROJECT-SPECIFIC REVIEW

Table 1 Summary of Scope of Program EIR

Action No.	Strategy	Tools and Techniques Analyzed in 2019 PEIR	Locations Analyzed in 2019 PEIR	Unit	Units per Year Analyzed in 2019 PEIR
MA-20	Retreat fuels in existing fuelbreaks	Pile burning; Cutting (manual and mechanical); Pulling (manual); Covering (mulching, chipping, and mastication)	All Fuelbreak zones	Acre	200
	Cyclical mowing of fine fuels		All Fuelbreak zones, with a focus on ignition prone areas: parking lots, picnic areas, and defensible space around structures	Acre	50
	Cyclical removal of broom in Optimized and Transitional Zones		Optimized and Transitional Fuelbreaks	Acre	260
	Roadside mowing		Service Roads	Acre	50
	Dam maintenance		Dams	Acre	50
MA-21	New fuelbreak construction	Pile burning; Cutting (manual and mechanical); Pulling (manual); Covering (mulching, chipping, and mastication)	New Fuelbreaks	Acre	15
MA-22	Annual surveys	Propane flaming; Cutting (manual and mechanical); Girdling (manual and mechanical); Pulling (manual and mechanical); Scalping (manual and mechanical); Covering (mulching, chipping, and mastication)	Optimized Fuelbreaks, Ecosystem Preservation Zone, Transitional Fuelbreaks, Ecosystem Restoration Zone, and Ecosystem Restoration/WAFRZ	Mile	150
	Weed control treatments		Optimized Fuelbreaks, Ecosystem Preservation Zone, Transitional Fuelbreaks, Ecosystem Restoration Zone, and Ecosystem Restoration/WAFRZ	Patch	100
MA-23	Initial reduction in accumulated fuels and brush	Broadcast burning; Pile burning; Cutting (manual and	Conifer and mixed hardwood forests adjacent to formal fuelbreaks, in Ecosystem Restoration/WAFRZ	Acre	60

Action No.	Strategy	Tools and Techniques Analyzed in 2019 PEIR	Locations Analyzed in 2019 PEIR	Unit	Units per Year Analyzed in 2019 PEIR
	Maintenance/ Planting	mechanical); Girdling (manual and mechanical); Pulling (manual and mechanical); Scalping (mechanical); Covering (mulching, chipping, and mastication); Planting (manual)	Ecosystem Restoration/WAFRZ	Acre	100
	Broadcast burning		Ecosystem Restoration/WAFRZ	Project	2
MA -24	Douglas-Fir thinning	Broadcast burning; pile burning; Cutting (manual and mechanical); Girdling (manual and mechanical); Pulling (manual and mechanical); Scalping (mechanical); Covering (solarization); Planting (manual)	Ecosystem Restoration Zone	Acre	200
	Broadcast burning for habitat and weed removal		Ecosystem Restoration Zone	Project	3
	Broom: Initial removal		Ecosystem Restoration Zone	Acre	300
	Broom: Long-term maintenance		Ecosystem Restoration Zone	Acre	205
	Goatgrass		Goatgrass at three locations within the Ecosystem Restoration/WAFRZ (see **Error! Reference source not found.** through **Error! Reference source not found.**)	Acre	35
	Yellow Starthistle		Yellow Starthistle within the Ecosystem Restoration Zone and Ecosystem Restoration/WAFRZ (see **Error! Reference source not found.** through **Error! Reference source not found.**)	Acre	120
	Other Priority Weeds		Optimized Fuelbreaks, Preservation Natural Areas, Transitional Fuelbreaks, Restoration Natural Areas, and Ecosystem Restoration/WAFRZ	Patch	-
MA-25	Planting	Broadcast burning; Pile burning; Propane	Ecosystem Restoration and Ecosystem Restoration/WAFRZ	Project	3

Action No.	Strategy	Tools and Techniques Analyzed in 2019 PEIR	Locations Analyzed in 2019 PEIR	Unit	Units per Year Analyzed in 2019 PEIR
	Habitat modification	flaming; Cutting (manual and mechanical); Girdling (manual and mechanical); Pulling (manual and mechanical); Covering (mulching, chipping, and mastication); Planting (manual)	Ecosystem Restoration and Ecosystem Restoration/WAFRZ	Project	3
MA-26	Restoration Plans	Broadcast burning; Pile burning; Propane flaming; Cutting (manual and mechanical); Girdling (manual and mechanical); Pulling (manual and mechanical); Scalping (mechanical); Covering (mulching, chipping, mastication, and solarization); Planting (manual)	--	--	3
MA-27	Implementation	Broadcast burning; Propane flaming; Cutting (manual and mechanical); Girdling (manual and mechanical); Pulling (mechanical); Scalping (manual and mechanical); Covering (mulching, chipping, mastication, and solarization); Grazing; Planting (manual)	Ecosystem Restoration and Ecosystem Restoration/WAFRZ	Project	

A PROJECT-SPECIFIC REVIEW

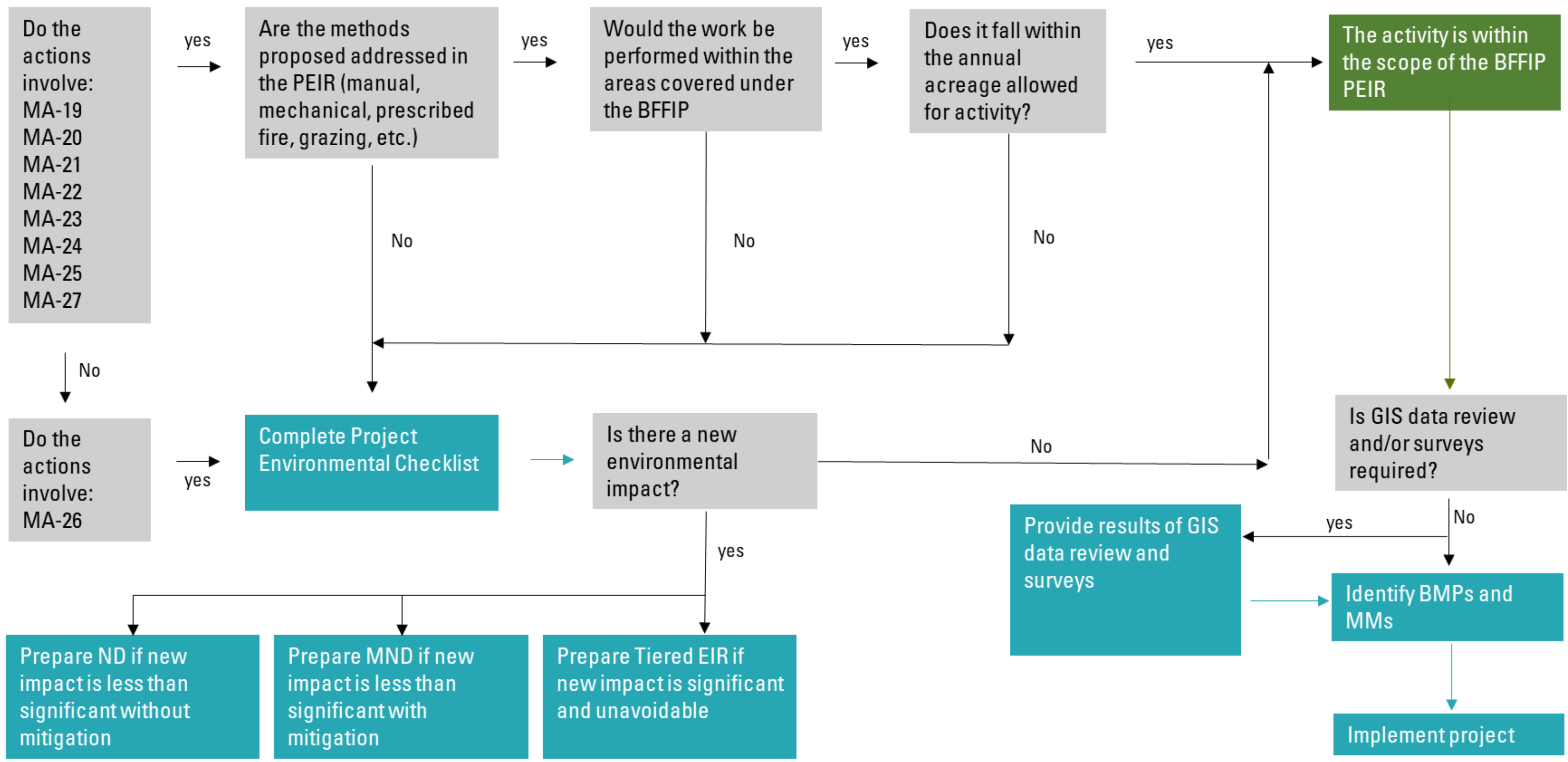
All MAs except for MA-26 that encompasses restoration plans, were analyzed in detail in the Program EIR. In most circumstances, work can be implemented without additional CEQA review. If work falls outside the parameters studied in the Program EIR, additional environmental review may be required.

For any work proposed, an initial screening review can be used to determine whether the environmental effects of the work were adequately analyzed in the Program EIR. The District will compare the proposed vegetation management actions against the activities, locations, types of tools and techniques, and units analyzed for each management action in the Program EIR.

If the proposed project does not fall within the scope of the analyzed management actions, the District will conduct an evaluation under a Project Environmental Checklist (PEC) (Chapter 2) to determine whether any new impacts could occur. Identification of new impacts will require further environmental review under CEQA. The type of review will be dependent upon the severity of the new impact. The flowchart in Figure 1 guides the process. The Project-Specific Screening Results Form and the Determination will be completed and saved with the Annual Work Plan. The PEC will be attached, if applicable. If the work is determined to be within the scope of the management actions proposed under the BFFIP, the appropriate best management practices (BMPs) and mitigation measures (MMs) will be identified and implemented (Chapter 3).

A PROJECT-SPECIFIC REVIEW

Figure 1 Flow Chart for Determining a Within the Scope of the BFFIP Finding or if Additional Environmental Review is Required



PROJECT-SPECIFIC SCREENING RESULTS FORM

What MAs would the actions involve?		
List Management Actions		
Are the methods proposed addressed in the Program EIR?	**Yes**	**No**
List methods		
Would the work be performed in areas covered under the Program EIR?	**Yes**	**No**
List locations of work		
Does the work fall within the acreage or units allowed for the year?	**Yes**	**No**
Identify units/acreages		
If the actions involve any MA but MA-26 and the answers to all questions above are "yes" – the actions are within the scope of the BFFIP Program EIR – go to the ***Determination Form***		
If the action involves MA-26 or the answer to any of the above questions is "no" – ***Complete the PEC and then complete the Determination Form***		

DETERMINATION FORM

On the basis of this initial evaluation:

	I find that all of the effects of the proposed project (a) have been analyzed adequately in the BFFIP Program EIR, (b) have been avoided or mitigated pursuant to the BFFIP Program EIR, and (c) all applicable mitigation measures and BMPs identified in the BFFIP Program EIR will be implemented. The proposed project is therefore **WITHIN THE SCOPE** of the BFFIP Program EIR. **NO ADDITIONAL CEQA DOCUMENTATION** is required.
	I find that the proposed project will have effects that were not examined in the BFFIP Program EIR. These effects are less than significant without any mitigation beyond what is already required pursuant to the BFFIP Program EIR. A **NEGATIVE DECLARATION** will be prepared.
	I find that the proposed project will have effects that were not examined in the BFFIP Program EIR. Although these effects might be significant in the absence of additional mitigation beyond what is already required pursuant to the BFIPP Program EIR, additional mitigation measures have been identified that would avoid or reduce the effects so that clearly no significant effects would occur. A **MITIGATED NEGATIVE DECLARATION** will be prepared
	I find that the proposed project will have environmental effects that were not examined in the BFFIP Program EIR. Because these effects are or may be significant and cannot be clearly mitigated, an **ENVIRONMENTAL IMPACT REPORT** will be prepared.

Signature Date

Printed Name Title

B. PROJECT ENVIRONMENTAL CHECKLIST

If any portion of the project (tools and techniques, locations, and units) is not within the scope of the Program EIR, per the flowchart in Figure 1 and as indicated on the Project-Specific Screening Results Form, the District will complete a PEC, the template for which is provided below.

The environmental resource areas included in the PEC are the same environmental resource analyzed in Chapter 3 of the Program EIR. The District will review the environmental analysis and mitigation measures in the Program EIR for each corresponding resource area in the Project Environmental Checklist. The District shall consider whether required BMPs and MMs would be effective in reducing or mitigating environmental impacts of the project considering the specific project activities and site-specific characteristics of the project area. Written explanations supporting all conclusions should be provided in the sections of the checklist available for discussion following the checklist questions presented for each resource area.

B.1 PROJECT INFORMATION

Project Title/Year of Implementation:	
Contact Person and Phone Number: (Provide phone number and email address)	
Project Location(s):	
Total Area to be Treated (acres):	
Description of Project: (Describe the whole action involved, including but not limited to later phases (e.g., maintenance) of the project, and any secondary, support, or off-site features necessary for its implementation. Attach additional sheets if necessary.)	
Treatment Tools and Techniques:	
Surrounding Land Uses and Setting: (Briefly describe the Project's surroundings)	
Other public agencies whose approval is required: (note status of any required approvals (permits))	
Native American Consultation. Pursuant to PRC Sections 21080.3.1, 21080.3.2, and 21082.3, lead agencies undertaking CEQA review must, upon written request of a California Native American tribe, begin consultation before the release of an environmental impact report, negative declaration, or mitigated negative declaration. For treatment projects that require additional CEQA review and documentation, have California Native American tribes traditionally and culturally affiliated with the project area requested consultation pursuant to Public Resources Code section 21080.3.1? If so, is there a plan for consultation that includes, for example, the determination of significance of impacts to tribal cultural resources, procedures regarding confidentiality, etc.? Note: For treatment projects that are within the scope of this PEIR, AB 52 consultation has been completed. The Board of Forestry and Fire Protection and CAL FIRE completed consultation pursuant to Public Resources Code section 21080.3.1 in preparation of the PEIR.	
Applicable Environmental Protection Measures. (Refer to Section 4)	

B.2 EVALUATION OF ENVIRONMENTAL IMPACTS

1. A brief explanation is required for all answers. Answers should consider whether the proposed project would result in new or more substantial environmental effects than described in the BFFIP Program EIR, after incorporation of applicable Environmental Protection Measures required by the BFFIP Program EIR.
2. All answers must take account of the whole action involved, including off-site as well as on-site, cumulative as well as project-level, indirect as well as direct, and short-term as well as long-term impacts.
3. Refer to the applicable resource analysis section in the BFFIP Program EIR for each environmental topic. If, after considering the specific location and characteristics of the proposed project, the project proponent determines that the proposed project would not result in new or more substantial environmental effects, then the checklist should indicate "No New Impact".
4. Once the project proponent has determined that a new or more substantial environmental effect may occur, then the checklist answers must indicate whether the impact is potentially significant, less than significant with mitigation, or less than significant without the need for mitigation. "Potentially Significant Impact" is appropriate if there is substantial evidence that an effect may be significant. If there are one or more "Potentially Significant Impact" entries when the determination is made, an EIR would be required.
5. Where a Negative Declaration, Mitigated Negative Declaration is required, the environmental review would be guided by the directions for use of the Program EIR with later activities in Section 15168. Where an EIR is required, the environmental review would be guided by Sections 15162 and 15163. When preparing any environmental document, the environmental analysis may incorporate by reference the analysis from the BFFIP Program EIR and focus the environmental analysis solely on issues that were not addressed in the BFFIP Program EIR.
6. Project proponents should incorporate into the environmental checklist references to information sources for potential impacts. Include a list of references cited in the environmental checklist and make copies of such references available to the public upon request.

B.3 AESTHETICS

Impact Statement	New Impact that is Significant or Potentially Significant	New Impact that is Less than Significant with Mitigation Incorporated	New Impact that is Less Than Significant Impact	No New Impact
Impact Aesthetics-1: The proposed project could have a substantial adverse effect on scenic vista and/or substantially degrade the existing visual character or quality of the non-urbanized site and its surroundings (public views are those that are experienced from publicly accessible vantage point) and the associated recreational experience.	☐	☐	☐	☐

B.3.1 Discussion

The discussion should identify which impacts from the Program EIR would occur from implementation of the proposed vegetation management project, describe the significance of each relevant impact and identify each mitigation measure from the Program EIR that is relevant to the proposed project. Additionally, this discussion should describe how each measure will address site-specific conditions and reduce impacts of the proposed vegetation management project.

B.4 AIR QUALITY

Impact Statement	New Impact that is Significant or Potentially Significant	New Impact that is Less than Significant with Mitigation Incorporated	New Impact that is Less Than Significant Impact	No New Impact
Impact Air-1: The proposed project could result in a cumulatively considerable net increase of any criteria pollutant for which the project region is in nonattainment under an applicable federal or state ambient air quality standard.	☐	☐	☐	☐
Impact Air-2: The proposed project could expose sensitive receptors to substantial pollutant concentrations.	☐	☐	☐	☐
Impact Air-3: The proposed project could conflict with or obstruct implementation of the applicable air quality plan.	☐	☐	☐	☐

B.4.1 Discussion

The discussion should identify which impacts from the Program EIR would occur from implementation of the proposed vegetation management project, describe the significance of each relevant impact and identify each mitigation measure from the Program EIR that is relevant to the proposed project. Additionally, this discussion should describe how each measure will address site-specific conditions and reduce impacts of the proposed vegetation management project.

B.5 BIOLOGICAL RESORUCES

Impact Statement	New Impact that is Significant or Potentially Significant	New Impact that is Less than Significant with Mitigation Incorporated	New Impact that is Less Than Significant Impact	No New Impact
Impact Biology-1: The proposed project could have a substantial adverse effect, either directly or through habitat modifications, on any species identified as a candidate, sensitive, or special-status species in local or regional plans, policies, or regulations, or by the California Department of Fish and Wildlife or the U.S. Fish and Wildlife Service.	☐	☐	☐	☐
Impact Biology-2: The proposed project could have a substantial adverse effect on any riparian habitat or other sensitive natural community identified in local or regional plans, policies, or regulations or by the California Department of Fish and Wildlife or the U.S. Fish and Wildlife Service.	☐	☐	☐	☐
Impact Biology-3: The proposed project could have a substantial adverse effect on State or federally protected wetlands (including, but not limited to, marsh, vernal pool, coastal, etc.) through direct removal, filling, hydrological interruption, or other means.	☐	☐	☐	☐
Impact Biology-4: The proposed project could interfere substantially with the movement of any native resident or migratory wildlife corridors, or impede the use of native wildlife nursery sites.	☐	☐	☐	☐

B.5.1 Discussion

The discussion should identify which impacts from the Program EIR would occur from implementation of the proposed vegetation management project, describe the significance of each relevant impact and identify each mitigation measure from the Program EIR that is relevant to the proposed project. Additionally, this discussion should describe how each measure will address site-specific conditions and reduce impacts of the proposed vegetation management project.

B.6 CULTURAL AND TRIBAL CULTURAL RESOURCES

Impact Statement	New Impact that is Significant or Potentially Significant	New Impact that is Less than Significant with Mitigation Incorporated	New Impact that is Less Than Significant Impact	No New Impact
Impact Cultural Resources-1: The proposed project could cause a substantial adverse change in the significance of a historical or archaeological resource pursuant to CEQA Guidelines Section 15064.5.	☐	☐	☐	☐
Impact Cultural Resources-2: The proposed project could disturb human remains, including those interred outside of formal cemeteries.	☐	☐	☐	☐
Impact Cultural Resources-3: The proposed project could cause a substantial adverse change in the significance of a tribal cultural resource as defined in PRC §21074 as either a site, feature, place, cultural landscape that is geographically defined in terms of the size and scope of the landscape, sacred place, or object with cultural value to a California Native American tribe, and that is: i. Listed or eligible for listing in the California Register of Historical Resources, or in a local register of historical resources as defined in PRC §5020.1(k), or j. A resource determined by the lead agency, in its discretion and supported by substantial evidence and with consideration of the significance of the resource to a California Native American tribe, to be significant pursuant to criteria set forth in subdivision (c) of PRC §5024.1.	☐	☐	☐	☐
Impact Cultural Resources-4: The proposed project could directly or indirectly destroy a unique paleontological resource or site or unique geologic feature.	☐	☐	☐	☐

B.6.1 Discussion

The discussion should identify which impacts from the Program EIR would occur from implementation of the proposed vegetation management project, describe the significance of each relevant impact and identify each mitigation measure from the Program EIR that is relevant to the proposed project.

Additionally, this discussion should describe how each measure will address site-specific conditions and reduce impacts of the proposed vegetation management project.

B.7 GEOLOGY AND SOILS

Impact Statement	New Impact that is Significant or Potentially Significant	New Impact that is Less than Significant with Mitigation Incorporated	New Impact that is Less Than Significant Impact	No New Impact
Impact Geology and Soils-1: The proposed project could result in substantial soil erosion or the loss of topsoil.	☐	☐	☐	☐
Impact Geology and Soils-2: The proposed project could result in substantial landslides or slope instability that could cause damage to important infrastructure or habitats in the water shed.	☐	☐	☐	☐

B.7.1 Discussion

The discussion should identify which impacts from the Program EIR would occur from implementation of the proposed vegetation management project, describe the significance of each relevant impact and identify each mitigation measure from the Program EIR that is relevant to the proposed project. Additionally, this discussion should describe how each measure will address site-specific conditions and reduce impacts of the proposed vegetation management project.

B.8 GREENHOUSE GASES

Impact Statement	New Impact that is Significant or Potentially Significant	New Impact that is Less than Significant with Mitigation Incorporated	New Impact that is Less Than Significant Impact	No New Impact
Impact GHG-1: The proposed project could generate greenhouse gas emissions that may have a significant impact on the environment.	☐	☐	☐	☐
Impact GHG-2: The proposed project could conflict with an applicable plan, policy, or regulation adopted for the purpose of reducing the emission of greenhouse gases.	☐	☐	☐	☐
Impact GHG-3: The proposed project could substantially decrease the overall ability of District Lands in the plan area to sequester carbon.	☐	☐	☐	☐

B.8.1 Discussion

The discussion should identify which impacts from the Program EIR would occur from implementation of the proposed vegetation management project, describe the significance of each relevant impact and identify each mitigation measure from the Program EIR that is relevant to the proposed project. Additionally, this discussion should describe how each measure will address site-specific conditions and reduce impacts of the proposed vegetation management project.

B.9 HAZARDOUS MATERIALS AND FIRE HAZARDS

Impact Statement	New Impact that is Significant or Potentially Significant	New Impact that is Less than Significant with Mitigation Incorporated	New Impact that is Less Than Significant Impact	No New Impact
Impact Hazards-1: The proposed project could compromise the health of individuals or create a significant hazard to the environment through emission of or exposure to hazardous materials.	☐	☐	☐	☐
Impact Hazards-2: The proposed project could create a significant hazard to the public, workers, or environment from contamination on-site or nearby at an existing hazardous materials site pursuant to Government Code Section 65962.5.	☐	☐	☐	☐
Impact Hazards-3: The proposed project could impair the implementation of or physically interfere with an adopted emergency response plan or emergency evacuation plan.	☐	☐	☐	☐
Impact Hazards-4: The proposed project could expose people or structures, either directly or indirectly, to a significant risk of loss, injury, or death involving wildland fires.	☐	☐	☐	☐
Impact Hazards-5: Due to slope, prevailing winds, and other factors, the proposed project could exacerbate wildfire risks and expose project occupants to, pollutant concentrations from a wildfire or the uncontrolled spread of a wildfire.	☐	☐	☐	☐
Impact Hazards-6: The proposed project could require the installation or maintenance of associated infrastructure (such as roads, fuelbreaks, emergency water sources, power lines or other utilities) that may exacerbate fire risk or that may result in temporary or ongoing impacts to the environment.	☐	☐	☐	☐
Impact Hazards-7: The proposed project could expose people or structures to significant risks, including downslope or downstream flooding or landslides, as a result of runoff, post-fire slope instability, or drainage changes.	☐	☐	☐	☐

B.9.1 Discussion

3 PROJECT ENVIRONMENTAL CHECKLIST

The discussion should identify which impacts from the Program EIR would occur from implementation of the proposed vegetation management project, describe the significance of each relevant impact and identify each mitigation measure from the Program EIR that is relevant to the proposed project. Additionally, this discussion should describe how each measure will address site-specific conditions and reduce impacts of the proposed vegetation management project.

B.10 HYDROLOGY AND WATER QUALITY

Impact Statement	New Impact that is Significant or Potentially Significant	New Impact that is Less than Significant with Mitigation Incorporated	New Impact that is Less Than Significant Impact	No New Impact
Impact Hydrology-1: The proposed project could violate any water quality standards or waste discharge requirements or otherwise substantially degrade surface or ground water quality, or substantially alter the existing drainage pattern of the sire or area, including through the alteration of the course of a stream or river or through the addition of impervious surfaces, in a manner which would result in substantial erosion or siltation on or off site.	☐	☐	☐	☐
Impact Hydrology-2: The proposed project could substantially alter the existing drainage pattern of the site or area, including through the alteration of the course of a stream or river or through the addition of impervious surfaces, in a manner which would: • substantially increase the rate or amount of surface runoff in a manner which would result in flooding on- or offsite; • create or contribute runoff water which would exceed the capacity of existing or planned stormwater drainage systems or provide substantial additional sources of polluted runoff; or • impede or redirect flood flows.	☐	☐	☐	☐
Impact Hydrology-3: The proposed project could conflict with or obstruct implementation of a water quality control plan or sustainable groundwater management plan.	☐	☐	☐	☐

B.10.1 Discussion

The discussion should identify which impacts from the Program EIR would occur from implementation of the proposed vegetation management project, describe the significance of each relevant impact and identify each mitigation measure from the Program EIR that is relevant to the proposed project. Additionally, this discussion should describe how each measure will address site-specific conditions and reduce impacts of the proposed vegetation management project.

B.11 NOISE

Impact Statement	New Impact that is Significant or Potentially Significant	New Impact that is Less than Significant with Mitigation Incorporated	New Impact that is Less Than Significant Impact	No New Impact
Impact Noise-1: The proposed project could generate a substantial temporary or periodic increase in ambient noise levels in the plan vicinity above levels existing without the project in excess of standards established in local General Plan or noise ordinance, or applicable standards of other agencies.	☐	☐	☐	☐

B.11.1 Discussion

The discussion should identify which impacts from the Program EIR would occur from implementation of the proposed vegetation management project, describe the significance of each relevant impact and identify each mitigation measure from the Program EIR that is relevant to the proposed project. Additionally, this discussion should describe how each measure will address site-specific conditions and reduce impacts of the proposed vegetation management project.

B.12 RECREATION

Impact Statement	New Impact that is Significant or Potentially Significant	New Impact that is Less than Significant with Mitigation Incorporated	New Impact that is Less Than Significant Impact	No New Impact
Impact Recreation-1: The proposed project could substantially degrade recreational experiences.	☐	☐	☐	☐

B.12.1 Discussion

The discussion should identify which impacts from the Program EIR would occur from implementation of the proposed vegetation management project, describe the significance of each relevant impact and identify each mitigation measure from the Program EIR that is relevant to the proposed project. Additionally, this discussion should describe how each measure will address site-specific conditions and reduce impacts of the proposed vegetation management project.

B.13 TRANSPORTATION AND TRAFFIC

Impact Statement	New Impact that is Significant or Potentially Significant	New Impact that is Less than Significant with Mitigation Incorporated	New Impact that is Less Than Significant Impact	No New Impact
Impact Transportation-1: The proposed project could conflict with or be inconsistent with CEQA Guidelines section 15064.3, subdivision (b).	☐	☐	☐	☐
Impact Transportation-2: The proposed project could substantially increase hazards due to a design feature (e.g., sharp curves or dangerous intersections) or incompatible uses (e.g., farm equipment).	☐	☐	☐	☐
Impact Transportation-3: The proposed project could result in inadequate emergency access.	☐	☐	☐	☐

B.13.1 Discussion

The discussion should identify which impacts from the Program EIR would occur from implementation of the proposed vegetation management project, describe the significance of each relevant impact and identify each mitigation measure from the Program EIR that is relevant to the proposed project. Additionally, this discussion should describe how each measure will address site-specific conditions and reduce impacts of the proposed vegetation management project.

B.14 ENERGY

Impact Statement	New Impact that is Significant or Potentially Significant	New Impact that is Less than Significant with Mitigation Incorporated	New Impact that is Less Than Significant Impact	No New Impact
Impact Energy-1: The proposed project could result in potentially significant environmental impact due to the wasteful, inefficient and unnecessary consumption or energy resources, during project construction or operation.	☐	☐	☐	☐
Impact Energy-2: The proposed project could conflict with or obstruct a state or local plan for renewable energy or energy efficiency.	☐	☐	☐	☐
Impact Energy-3: The proposed project could result in a substantial increase in demand upon energy resources in relation to projected supplies.	☐	☐	☐	☐
Impact Energy-4: The proposed project could result in longer overall distances between jobs and housing.	☐	☐	☐	☐

B.14.1 Discussion

The discussion should identify which impacts from the Program EIR would occur from implementation of the proposed vegetation management project, describe the significance of each relevant impact and identify each mitigation measure from the Program EIR that is relevant to the proposed project. Additionally, this discussion should describe how each measure will address site-specific conditions and reduce impacts of the proposed vegetation management project.

C. APPLICABLE ENVIRONMENTAL PROTECTION MEASURES

The section identifies the surveys and GIS review and the environmental protection measures that are applicable to the proposed activities. These measures take the form of best management practices (BMPs) and Mitigation Measures (MMs). Some BMPs and MMs apply to all projects, while others only apply to projects that include specific treatment types, treatment activities, or locations. Table 2, below, provides a comprehensive list of BMPs and MMs applicable to each project type. MMWD shall verify that all applicable BMPs and MMs will be implemented. Refer to the Mitigation Monitoring and Reporting Plan for entity responsible for implementing and verifying or enforcing each measure. The applicable measures are shown with a checkmark. The form identifying the mitigation measures should be completed for each activity identified in the Annual Work Plan.

Table 2 Applicable Environmental Protection Measures Matrix

	Tool/Technique																
		Prescribed Burning			Cutting			Girdling	Pulling		Scalping			Covering			Planting
BMPs and MMs	Access and Vehicle Travel	Broadcast burning	Pile burning	Propane Flaming	Cutting with heavy equipment	Cutting with power hand tools	Cutting with non-power hand tools	Manual and Mechanical	Pulling with heavy equipment	Pulling by hand or with non-power tools	Scalping with heavy equipment	Scalping with power tools	Scalping with hand tools	Mulching/ Chipping/ Mastication[a]	Solarization	Grazing	Manual
Entire Plan Area																	
BMP-1	√				√				√		√						
BMP-2	√				√				√		√						
BMP-3	√				√				√		√						√
BMP-4																	√
BMP-5	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
BMP-6																	√
BMP-7					√	√	√	√	√	√	√	√	√	√			√
MM Air-1		√															
MM Air-2	√				√				√	√	√						√
MM Air-3		√	√														
MM Air-4		√	√														
MM Biology-1	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
MM Biology-2	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
MM Biology-3	√	√			√	√	√	√	√	√	√	√	√	√	√		
MM Biology-4	√				√	√		√	√		√	√		√			
MM Biology-7	√	√	√		√	√	√		√		√			√			
MM Cultural-1		√	√		√				√		√			√			
MM Cultural-2		√	√		√				√		√			√			
MM Cultural-3		√	√		√				√		√			√			√
MM Cultural-4									√	√							√
MM Hazards-1	√				√	√		√√	√		√	√		√			
MM Hazards-3			√														
MM Hazards-4		√															
MM Hazards-6				√													
MM Hazards-7	√				√				√		√			√			
MM Hydrology-1	√				√				√		√						
MM Geology-1		√			√				√	√	√			√		√	√

	Tool/Technique																
		Prescribed Burning			Cutting			Girdling	Pulling		Scalping			Covering			Planting
BMPs and MMs	Access and Vehicle Travel	Broadcast burning	Pile burning	Propane Flaming	Cutting with heavy equipment	Cutting with power hand tools	Cutting with non-power hand tools	Manual and Mechanical	Pulling with heavy equipment	Pulling by hand or with non-power tools	Scalping with heavy equipment	Scalping with power tools	Scalping with hand tools	Mulching/ Chipping/ Mastication[a]	Solarization	Grazing	Manual
MM Geology-2		√															
MM Geology-3																√	
MM Noise-1	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Stream, Riparian, and Wetland Habitats																	
MM Biology-15	√				√				√		√						
MM Biology-17	√	√	√		√				√		√						
Bat Roosting Habitat																	
MM Biology-5	√	√	√		√	√		√	√		√	√		√			
Badger Denning Habitat																	
MM Biology-6	√	√	√		√				√		√			√		√	
Northern Spotted Owl Habitat and Activity Centers																	
MM Biology-8	√	√	√		√	√	√	√	√	√	√	√	√	√	√		√
MM Biology-14	√	√	√	√	√	√	√	√	√	v	√	√	√	√	√	√	√
Western Pond Turtle Habitat																	
MM Biology-9	√	√	√		√				√		√			√			
California Red-Legged Frog Habitat																	
MM Biology-10	√	√	√		√				√		√			√			
Stonecrop Habitat																	
MM Biology-11	√	√	√		√				√	√	√			√			√
Big Carson Creek, Little Carson Creek, and Their Tributaries																	
MM Biology-12	√	√	√		√				√		√			√			
Seeps, Springs, and Potrero Meadow																	
MM Biology-13	√	√	√		√	√		√	√		√			√	√	√	
Grassland Habitat																	
MM Biology-16	√				√				√		√			√		√	
Mill Valley Air Force Station																	
MM Hazards-2	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Roads and Trails																	
MM Hazards-5		√															

3 APPLICABLE ENVIRONMENTAL PROTECTION MEASURES

	Tool/Technique																
	Prescribed Burning				Cutting			Girdling	Pulling		Scalping			Covering			Planting
BMPs and MMs	Access and Vehicle Travel	Broadcast burning	Pile burning	Propane Flaming	Cutting with heavy equipment	Cutting with power hand tools	Cutting with non-power hand tools	Manual and Mechanical	Pulling with heavy equipment	Pulling by hand or with non-power tools	Scalping with heavy equipment	Scalping with power tools	Scalping with hand tools	Mulching/ Chipping/ Mastication[a]	Solarization	Grazing	Manual
MM Recreation-1	√				√				√		√			√			
MM Transportation-1	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√

Notes:

[a] In the Program EIR analysis, the use of mechanical equipment to masticate, chip, or mulch vegetation for use under the covering technique was analyzed under "mechanical techniques for vegetation removal".

LIST OF MITIGATION MEASURES APPLICABLE TO ACTION OR ACTIVITIES

MAs to be performed

Tools/techniques to be utilized

Locations of work

List of Applicable BMPs and MMs

3 APPLICABLE ENVIRONMENTAL PROTECTION MEASURES

Results of surveys and GIS review, if applicable and location specific considerations

APPENDIX B:
COMMENT LETTER B11 SUBMITTED STUDIES

Rangeland Ecol Manage 63:128–136 | January 2010 | DOI: 10.2111/REM-D-09-00106.1

Soil Carbon Pools in California's Annual Grassland Ecosystems

Whendee L. Silver,[1] *Rebecca Ryals,*[2] *and Valerie Eviner*[3]

Authors are [1]Professor and [2]graduate student, Ecosystem Sciences Division, Department of Environmental Science, Policy, and Management, 137 Mulford Hall No. 3114, University of California, Berkeley, CA 94720, USA; and [3]Assistant Professor, Department of Plant Sciences, University of California–Davis, 1210 PES, Mail Stop 1, One Shields Ave, Davis, CA 95616, USA.

Abstract

Rangeland ecosystems cover approximately one-third of the land area in the United States and half of the land area of California. This large land area, coupled with the propensity of grasses to allocate a considerable proportion of their photosynthate belowground, leads to high soil carbon (C) sequestration potential. Annual grasslands typical of the Mediterranean climates of the western United States differ in their life history strategies from the well-studied perennial grasslands of other regions and thus may also differ in their soil C pools and fluxes. In this study we use the literature to explore patterns in soil C storage in annual grass-dominated rangelands in California. We show that soil C is highly predictable with depth. Cumulative soil C content increased to 2–3-m depth in rangelands with a woody component and to at least 1-m depth in open rangelands. Soil C within a given depth varied widely, with C content in the top 1-m depth spanning almost 200 Mg $C \cdot ha^{-1}$ across sites. Soil C pools were not correlated with temperature or precipitation at a regional scale. The presence of woody plants increased C by an average of 40 $Mg \cdot ha^{-1}$ in the top meter of soil. Grazed annual grasslands had similar soil C content as ungrazed grassland at all depths examined, although few details on grazing management were available. Soil C pools were weakly positively correlated with clay content and peaked at intermediated levels of aboveground net primary production. Our results suggest that annual grasslands have similar soil C storage capacity as temperate perennial grasslands and offer an important resource for mitigation of greenhouse gas emissions and climate change.

Resumen

Los ecosistemas de pastizales naturales cubren aproximadamente un tercio del área territorial de los EE. UU., y la mitad del área de California. Esta gran superficie, sumada a la propensión de los pastos a asignar una proporción considerable de sus fotosintatos a estructuras radiculares resulta en un alto potencial de secuestro de carbono (C). Los pastizales de especies anuales típicos de los climas Mediterráneos del oeste de los EE.UU. presentan estrategias de historias de vida diferentes de los pastizales perennes de otras regiones que han sido bien estudiados, y por lo tanto podrían también presentar diferencias en los flujos y reservas de carbono del suelo. En este estudio nos basamos en la literatura para explorar los patrones de almacenamiento de carbono en el suelo en pastizales dominados por especies anuales en California. Demostramos que el C en el suelo es altamente predecible a partir de la profundidad del suelo. El contenido acumulado de C en el suelo aumentó hasta los 2–3 m de profundidad en pastizales con un componente leñoso y hasta por lo menos 1m de profundidad en pastizales abiertos. El contenido de C del suelo a una determinada profundidad varió ampliamente, con el C del primer metro de profundidad oscilando en casi 200 Mg $C \cdot ha^{-1}$ a lo largo de los sitios muestreados. Las reservas de C del suelo no correlacionaron con temperatura o precipitación a escala regional. La presencia de plantas leñosas incrementó el C en el primer metro de profundidad del suelo en 40 $Mg \cdot ha^{-1}$ en promedio. Los pastizales anuales pastoreados tuvieron contenidos de C similares a los pastizales no pastoreados a todas las profundidades del suelo examinadas, aunque se encontraron pocos detalles sobre el manejo del pastoreo. Las reservas de C del suelo presentaron una correlación positiva débil con el contenido de arcillas, y fueron máximas en sitios con niveles intermedios de productividad primaria aérea neta. Nuestros resultados sugieren que los pastizales anuales presentan capacidades de almacenamiento de C similares a las de los pastizales de especies perennes de climas templados y ofrecen un recurso importante en la mitigación de la emisión de gases de invernadero y cambio climático.

Key Words: annual grassland, climate change, Mediterranean climate, oak woodland, soil carbon sequestration

INTRODUCTION

Soil carbon (C) sequestration has been proposed as a means to lower greenhouse gas concentrations and help offset emissions (Intergovernmental Panel on Climate Change [IPCC] 2007). C sequestration in soils is accomplished by increasing the amount and/or mean residence time of C stored belowground. Several approaches have been proposed for increasing rates of soil C sequestration, including reforestation and afforestation of cropland and rangeland (Post and Kwon 2000), use of conservation tillage or no-till agriculture (Six et al. 2000; Del Grasso et al. 2009), improved grazing practices (Conant et al.

This research was supported by a grant from the Environmental Defense Fund to W.L.S. Additional support came from the Kearney Foundation for Soil Science, the Rathmann Family Foundation, the Marin Community Foundation, and the California Agricultural Experiment Station (7673-MS to W.L.S.).

This publication was made possible through support provided to the Global Livestock Collaborative Research Support Program by the Office of Agriculture, Bureau for Economic Growth, Agriculture and Trade, United States Agency for International Development under terms of Grant No. PCE-G-00-98-00036-00. The opinions expressed herein are those of the author(s) and do not necessarily reflect the views of the USAID.

Correspondence: Whendee L. Silver, Ecosystem Sciences Division, Dept of Environmental Science, Policy, and Management, 137 Mulford Hall No. 3114, University of California, Berkeley, CA 94720, USA. Email: wsilver@berkeley.edu

Manuscript received 20 July 2009; manuscript accepted 8 November 2009.

2001), and organic amendments such as biochar (Lehmann 2007) and compost (Lal 2004; Smith 2004).

Rangeland soils are widely recognized for their potential to sequester C (Lal et al. 1995; Follett et al. 2001; Soussana et al. 2004; Smith et al. 2008), due in part to their extensive land area. Rangeland covers approximately 31% of the land area in the United States and an estimated 40–70% globally (Branson et al. 1981; Havstad et al. 2009). Furthermore, the relatively high ratio of evapotranspiration to precipitation in the grassland biome drives considerable belowground allocation by rangeland plants (Jackson et al. 1996). This high root biomass contributes to soil C pools directly through organic matter inputs and indirectly through increased soil aggregation and the formation of recalcitrant humic substances (Miller and Jastrow 1990; Balesdent and Balabane 1996).

Grazing management can affect soil C storage by altering above- and belowground plant C allocation (Holland et al. 1992; Johnson and Matchett 2001), net primary productivity (NPP; Frank and McNaughton 1993; Turner et al. 1993), and soil respiration (Kieft 1994; Bremer et al. 1998; Cao et al. 2004). Moderate grazing often increases aboveground NPP (ANPP; McNaughton 1985; Loeser et al. 2004), and in perennial grasslands ANPP is frequently positively correlated with soil C pools when controlling for precipitation (Parton et al. 1994).

Despite the well-recognized potential for rangelands to store C, there have been few regional surveys of soil C pools in rangeland ecosystems (Bronson et al. 2004; Derner and Schuman 2007; Smith et al. 2008). Regional-scale soil C analyses that include information on patterns in climate, soil type, cover type, or management allow us to explore the relative sensitivity of soil C pools to the environment and to management practices. This information can then be used to identify promising approaches and technologies for C sequestration. Most of our understanding of C sequestration potential in US rangelands is from the Great Plains (Follett et al. 2001; Schuman et al. 2002; Derner and Schuman 2007). Although these systems occur along a gradient of mean annual precipitation and temperature, they are restricted to temperate climatic conditions that differ greatly from the Mediterranean climates of the western United States. These differences in climate may lead to significant differences in the temporal and spatial patterns in C storage and loss.

Rangelands cover approximately 50% of the state of California, a land area of approximately 24 million ha (Brown et al. 2004). Broadly defined, these ecosystems are characterized as grasslands, scrub, and woodlands (californiarangeland.ucdavis.edu), but the primary source of forage is from areas dominated by annual grasses and forbs, open oak woodlands, and occasional patches of both native and non-native perennial grasses, particularly near the coast (George et al. 2001; Jackson and Bartolome 2002). The climate varies throughout the state (Holland and Kiel 1995), with increasing precipitation from south to north, and from inland to coastal environments.

California rangelands differ in several ways from the perennial temperate grasslands of the Midwestern United States. California's climate is characterized by cool wet winters and warm dry summers. These grasslands are dominated by annual grasses and forbs, which germinate in the mid- to late fall, grow slowly throughout the winter months, and increase growth rates in the spring. They flower and die in late spring to early summer, leaving the soil surface covered in a thick layer of surface litter until rains commence again in the fall (Heady et al. 1991). This life history strategy is likely to favor a lower root-shoot allocation and shallower rooting depth than perennial grasslands, as no active plant biomass occurs over the dry summer months (Heady et al. 1991). A low root-shoot ratio and shallow roots could lead to lower soil C storage relative to perennial grasslands.

In California's annual grassland, each season's peak aboveground biomass is equivalent to its ANPP. ANPP is partially influenced by temperature, precipitation, soils, and the amount of residual dry matter (RDM), the previous year's aboveground biomass remaining at the beginning of the growing season (Hedrick 1948; Heady 1956, 1965; George et al. 1985). Grazing management in annual grasslands is often based on this relationship between RDM and the ensuing year's productivity (George et al. 1985) and may feed back on soil C pools and fluxes. Aboveground NPP tends to peak at moderate to high levels of RDM (indicator of moderate to no grazing; Bartolome et al. 2007). In contrast, decreased RDM (indicator of increased grazing) leads to higher root:shoot ratios in these annual grasses (Betts 2003). Most temperate perennial grasses have been found to decrease root:shoot ratios in response to defoliation, highlighting that the different responses of annual vs. perennial grasses to grazing limit our ability to predict the impacts of grazing practices on these annual systems based on data from perennial grasslands (reviewed in Bartolome et al. 2007).

We have compiled data from peer-reviewed literature to estimate the quantity of C stored in rangeland soils in California and to explore the effects of climate, soil type, vegetation cover type, grazing, and ANPP. Our goals were to provide an estimate of current levels of soil C storage in California rangelands and to identify promising approaches for increased C sequestration in the future.

METHODS

We extracted data from peer-reviewed journal articles that reported sampling depth and soil organic C content, or sampling depth, soil C concentration, and bulk density from California rangelands (Table 1). Rangelands were defined broadly and included grasslands, oak savanna, oak woodland, coastal grassland complexes, and woody savannas. Only studies that included data on soil C pools with at least partial grass cover were included here ($n = 48$ separate soil profiles, 216 data points). Using the same publications or others from the same sites we collected information on soil order, latitude, longitude, mean annual temperature, mean annual precipitation, ANPP during the study period (ANPP of grass component), clay concentration (%), and grazing management (grazed, ungrazed). We also recorded the vegetation cover type (presence or absence of woody plants, herbaceous perennial plants, and nitrogen [N] fixers); however, insufficient data were available for meta-analyses of all variables except presence or absence of woody plants. Only profiles and depths reporting direct measurements (i.e., not modeled values) were used.

Table 1. Studies reporting soil C content in California rangelands.[1]

Study	Profile (no.)	Mean annual temperature (°C)	Mean annual precipitation (mm)	Latitude (°)	Longitude (°)
Dahlgren et al. (1997)	4	15	730	39.2	121.2
Herman et al. (2003)	3	15	730	39.2	121.2
Steenwerth et al. (2002)	4	14.25	425	NA	NA
Fierer et al. (2005)	6	22	500	34.4	120.0
Camping et al. (2002)	3	15	730	39.2	121.7
Zavaleta and Kettley (2006)	2	14.5	605	NA	NA
Waldrop and Firestone (2006)	1	15	940	NA	NA
Jackson et al. (1988)	1	15	730	39.2	121.2
Chou et al. (2008)	1	16	750	39.2	121.2
Trumbore et al. (1996)	1	17.8	310	36.7	119.3
Carbone et al. (2008)	1	24	150	37.4	118.4
Sanderman et al. (2008); Sanderman and Amundson (2008)	1	14	1 300	NA	NA
Baisden et al. (2002a, 2002b); Baisden and Parfitt (2007)	4	16	300	37.5	120.5
Gessler et al. (2000)	9	22	380	34.4	120.2
Masiello et al. (2004)	7	12	> 1 000	NA	NA

[1]NA indicates data not available.

Sampling depths ranged from 2 cm to 365 cm and varied widely within and among studies. Soil C content varies as a function of the depth to which soil is sampled, and for this reason some standardization is required to compare among sites where different soil depths are sampled. This approach is valid for regional scale analyses (Jobbagy and Jackson 2000; Silver et al. 2000, 2002) but is less appropriate for site-specific data. We used a simple modeling procedure to standardize depths across the 48 profiles to facilitate comparisons among studies (Jobbagy and Jackson 2000; Silver et al. 2000, 2002). Soil C pools were summed by depth to estimate cumulative soil C pools for every possible depth within a profile (e.g., for a profile that sampled 0–2-cm, 2–10-cm, and 10–30-cm depth we calculated C pools for 0–2-cm, 0–10-cm, and 0–30-cm depths). We then used the entire dataset to plot cumulative soil C pools with depth; we fit one curve to all the data, estimating the goodness of fit using regression analyses. In our first analysis we used all the data available, which allowed us to determine if patterns existed and identify any obvious statistical outliers, using analysis of variance (Systat 11; Systat Software Inc., Chicago, IL). Through this procedure, we identified eight profiles derived from a single study in northwestern California and one profile from central California as outliers. Outliers were removed and treated separately in all subsequent analyses and are discussed below (see Results). The remaining 39 profiles are hereafter referred to as primary profiles.

Using the primary profiles we again plotted cumulative soil C with depth and derived the following equation:

$$y = -2.79x^2 + 1\,725x + 13\,241 \qquad [1]$$

where y is the soil C pool at depth x ($r^2 = 0.90$, $n = 145$). We used Equation 1 to generate one modeled C value for each soil depth increment reported in the literature (e.g., 0–2 cm, 0–2.5 cm, 0–8 cm, etc.; $n = 80$). We then estimated soil C pools at target depths of 10 cm, 25 cm, 50 cm, and 100 cm for each profile by multiplying the measured C content at the depth from each profile that was closest to the target depth by a conversion factor. The conversion factor was the ratio of modeled C at the target depth (Eq. 1: $y(x)$ 10 cm, 25 cm, 50 cm, or 100 cm) to the C modeled at the nearest depth reported in the literature (Eq. 1 using the measured depth for x that was closest to the target depth). For example, cumulative C for a depth of 50 cm in a profile where the closest reported depth is 40 cm was calculated as: measured C at 40 cm $\cdot\, y(50)/y(40)$.

We used data modeled to the four depths to determine the effects of climate, grazing, and woody plants on soil C pools at the four depths. We also explored relationships of soil C with ANPP. Data were log transformed when appropriate to meet the assumptions of analysis of variance. Statistical analyses were performed using Systat 11. Statistical significance was determined at the 95% level unless otherwise noted. Values in the text are means ± 1 SE.

RESULTS

Cumulative soil C pools followed a predictable pattern with depth when using the primary soil profiles ($r^2 = 0.90$, $n = 39$ profiles; Fig. 1A). The pattern was surprisingly linear for the top 2 m and appeared to saturate only near 3 m in rangelands with a woody component. Rangelands without a woody component were sampled only to 1-m depth. Cumulative soil C pools were greatest in the top 20 cm but were relatively linear over most of this range. The outliers followed similar patterns but were generally offset from the primary profiles (Fig. 1B). One profile, reported in Gessler et al. (2000), had higher-than-average soil C content at each of eight depth increments (Fig. 1A). This profile was located in a small but deep valley/canyon at the confluence of two drainages. Surface soils had accumulated via runoff from the surrounding area,

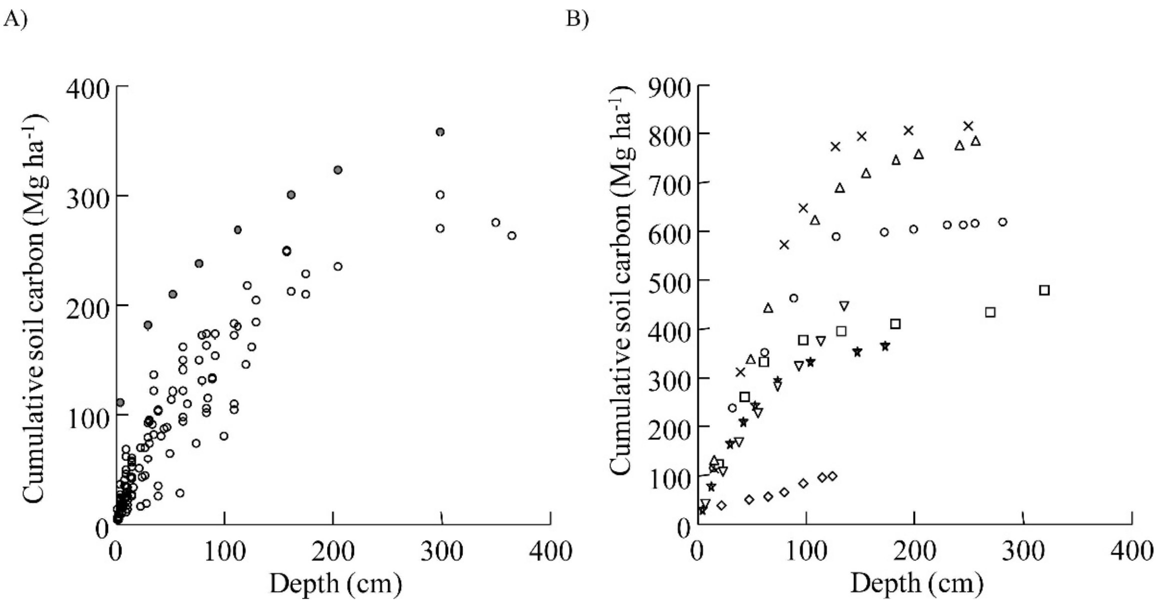


Figure 1. Cumulative carbon pools by depth in California rangeland soils. **A,** Primary profiles ($n = 40$); $y = -2.79x^2 + 1\,725x + 13\,241$; $r^2 = 0.90$; filled dots are from Gessler et al. (2000). **B,** Data from Masiello et al. (2004).

leading to a very C-rich profile (O. Chadwick, personal communication, May 2009). A set of profiles from a study conducted by Masiello et al. (2004) on the northern California coast did not follow the pattern of the primary profiles (Fig. 1B). The soil C measured in this study exceeded that of the other studies, with the exception of one profile in relatively recent soils (~ 3.9 thousand yr old). The high soil C content of these soils is intriguing and may result from previous land cover in forest, due to the presence of deep coarse roots found in some pits (O. Chadwick, personal communication, May 2009). Cumulative soil C pools reached an asymptote between 1-m depth and 2-m depth at this site.

There was a wide range in soil C pools at all modeled depths, and the range increased as more of the soil profile was considered (Table 2; Fig. 2). Soil C pools from the primary profiles ranged from 28 Mg $C \cdot ha^{-1}$ to 137 Mg $C \cdot ha^{-1}$ in the 0–50-cm depth and spanned 173 Mg $C \cdot ha^{-1}$ when considering the top meter of soil. There was an average of 90 ± 5 Mg $C \cdot ha^{-1}$ to 50-cm depth and 140 ± 7 Mg $C \cdot ha^{-1}$ to 1-m depth. The potential controlling factors most likely to mediate this variability that were available from the literature included soil type, clay content, climate, ANPP, and grazing history. Alfisols ($n = 18$) and Mollisols ($n = 14$) were the two dominant soil orders and had similar soil C content (data not shown). Although soil type did not significantly contribute to variation in soil C, soil C pools were positively correlated with clay content below 10-cm depth (Fig. 3). Mean annual temperature across sampling points ranged from 14°C to 24°C, and mean annual precipitation ranged between 150 $mm \cdot yr^{-1}$ and 1 300 $mm \cdot yr^{-1}$. There were no statistically significant trends in soil C pools with temperature or precipitation. Aboveground NPP was available for 19 profiles and varied from 129 $kg \cdot ha^{-1} \cdot yr^{-1}$ to $> 8\,000$ $kg \cdot ha^{-1} \cdot yr^{-1}$. Rates of ANPP were significantly greater above 500-mm mean annual precipitation and 20°C mean annual temperature, but much of the variation in ANPP was not explained by climate variables. Soil C content peaked at intermediate levels of ANPP ($y = -14.8x^2 + 201.7x - 55.6$, $r^2 = 0.44$, $P < 0.01$, $F = 6.3216$; 50-cm depth).

The presence of a woody component in rangelands significantly increased soil C pools for 0–25-cm, 0–50-cm, and 0–100-cm depths (Table 2). In the top meter of soil, woody plants increased soil C pools by approximately 40 Mg $C \cdot ha^{-1}$ (116 ± 9 vs. 155 ± 9 Mg $C \cdot ha^{-1}$ in nonwoody vs. woody rangelands). Grazed sites had slightly more soil C at all modeled depths than ungrazed ones, but the differences were small and statistically insignificant at this level of resolution (Table 2). It is important to note that grazing management likely varied among the studies with regard to history, duration, and intensity.

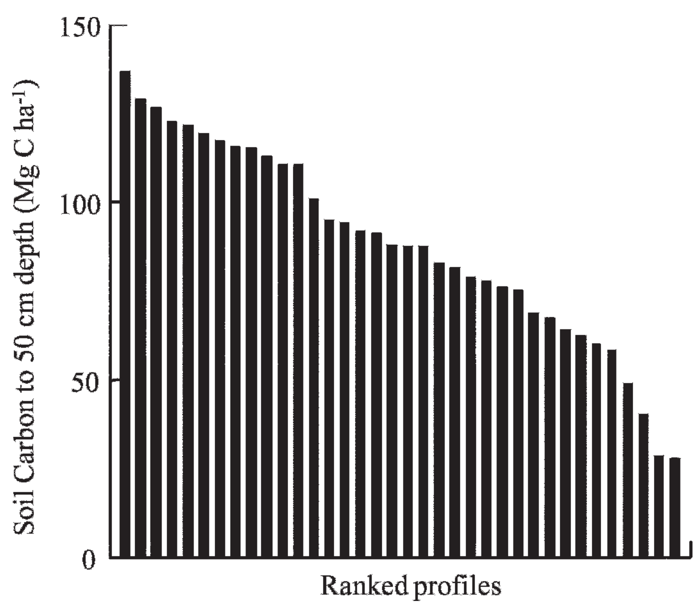


Figure 2. Modeled soil carbon to 50-cm depth (ranked from highest to lowest) for the primary profiles.

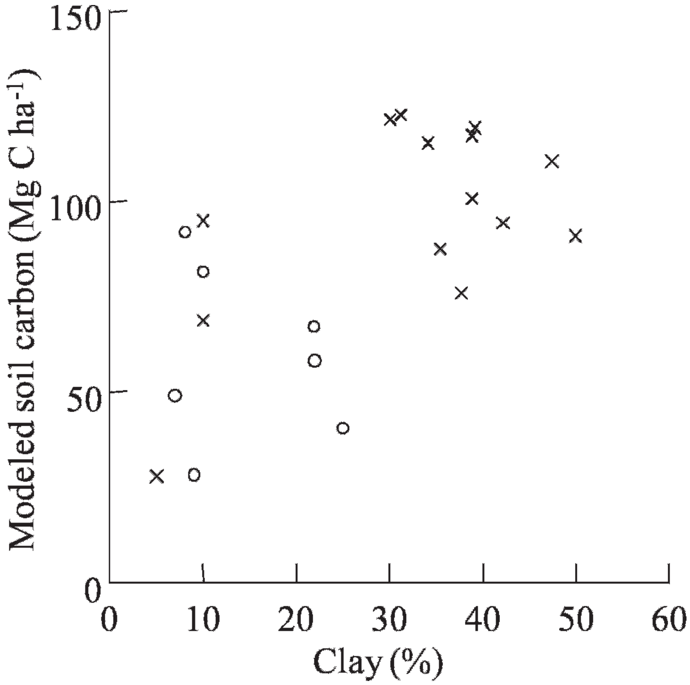


Figure 3. Modeled soil carbon content (Mg C · ha^{-1}) plotted against soil clay content (%); $r^2 = 0.27$, $P < 0.01$; X = rangeland with woody component; 0 = rangeland without woody component.

Published studies on soil C pools were concentrated at relatively few research sites throughout the state (Fig. 4). Eleven of the primary profiles were at the Sierra Foothills Research and Extension Center, Browns Valley, Yuba County (lat 39°15′N, long 121°17′W). Thirteen profiles were located at the University of California Sedgewick Natural Reserve in the Santa Ynez River Basin, Santa Barbara County (lat 34°42′N, long 120°03′W). Within site variability was relatively high at sites with multiple profiles; this is not surprising given that sampling locations were often selected to compare contrasting environmental conditions and treatments. Very few studies reporting soil C have been conducted in the more arid southern and southeastern portions of the state.

DISCUSSION

Regional Patterns in Soil Carbon Pools

Few regional surveys have been performed of soil C pools in rangeland ecosystems. Soil C pools in California rangelands were slightly larger than the average values for grasslands and pastures for the conterminous United States for the top 100 cm (Guo et al. 2006). Soil C pools in this study averaged 90 Mg C · ha^{-1} in the top 50 cm. This is lower than values reported for perennial grasslands in Texas, which stored approximately 100 Mg C · ha^{-1} in the top 40 cm of soils (Potter and Derner 2006). Burke et al. (1989) estimated soil C pools to 20-cm depth from 500 rangeland sites in the Great Plains using soil survey data. They reported soil C pools that ranged from less than 10 Mg C · ha^{-1} to almost 90 Mg C · ha^{-1} and were correlated with broad climate differences across the region. They found that cool, moist climates and clay soils led to the largest soil C pools. California's soil C pools in the top 20 cm averaged 50 ± 4 Mg C · ha^{-1}. These fall within the range found in the Great Plains, and when compared to sites in the Great Plains with similar averaged climate values (mean annual temperature was 19 ± 0.3°C and mean annual precipitation was 517 ± 16 mm), soil C pools in California were greater than

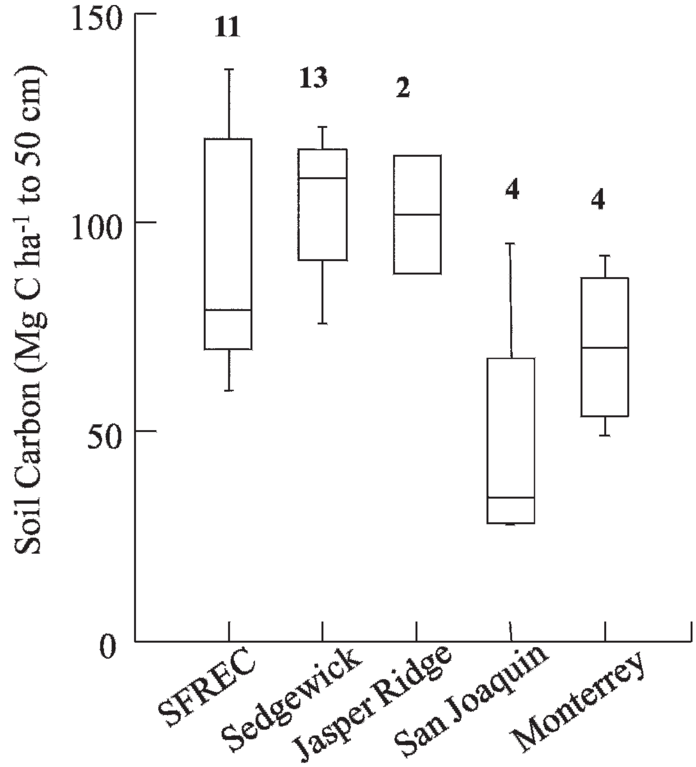


Figure 4. Box and whisker plots showing range of soil carbon data from the five dominant study sites. Numbers above each box indicate the number of profiles used from that site.

Table 2. Patterns in soil carbon pools (Mg C · ha^{-1}) with depth in California rangeland soils. Data are modeled using a simple quadratic equation with soil carbon content and depth (see text).[1]

Depth (cm)	Overall Mean ± SE	Overall Min/max	Woody (mean ± SE)	Nonwoody (mean ± SE)	Ungrazed (mean ± SE)	Grazed (mean ± SE)
0–10	33 ± 4	11/152	36 ± 7	27 ± 3	32 ± 5	34 ± 4
0–25	58 ± 4	16/158	66 ± 7	44 ± 4*	52 ± 7	57 ± 6
0–50	90 ± 5	28/200	101 ± 7	71 ± 6*	80 ± 9	89 ± 8
0–100	140 ± 7	47/246	155 ± 9	116 ± 9*	127 ± 12	141 ± 13

[1]Asterisks signify statistically significant differences among treatments (woody vs. nonwoody and ungrazed vs. grazed). Sample size (per depth) was 40 for overall data, 25 for woody rangelands, 15 for nonwoody sites, 9 for ungrazed, and 11 for grazed rangelands.

in Central Plains rangelands ($\sim$20 Mg C $\cdot$ ha^{-1}) for the same depth. Unlike the Great Plains, most rangelands in California rarely experience prolonged low temperatures, and most of the annual precipitation in California falls as rain during the growing season. This likely maximizes the use of precipitation by grasses, while during the warm summer months soil organic matter decomposition is likely inhibited due to lack of adequate soil moisture. We did not see a relationship between precipitation and soil C in California rangeland soils. Soil C pools increased with clay content in this study. Finely textured soils have greater reactive surface area and tend to store more C than more coarsely textured soils (Schimel et al. 1994). Soil C pools were greatest at intermediate levels of ANPP. This may result from patterns in plant allocation, with greater belowground allocation contributing proportionally more to soil C pools (Balesdent and Balabane 1996; Christian and Wilson 1999; Puget and Drinkwater 2001).

Our analyses show that the cumulative C content of rangeland soils in California was strongly predictable with depth. The strong relationship of soil C pools with depth suggests that although local properties may contribute to variability in the baseline amount of C in these soils, inherent properties of rangelands control patterns of C accumulation and storage over the soil profile. Although our dataset includes multiple profiles from a subset of sites, the studies used in this analysis spanned multiple bioclimatic regions (semiarid to mesic), soil types (alfisols and mollisols), cover types (annual grasslands and oak woodlands), and management (ungrazed and a range of grazing intensities). The trend in soil C accumulation with depth was nearly linear over 2 m in rangelands with a woody component and over 1 m in open rangelands. Most of the rangelands in this study were dominated by annual grasses, which are generally thought to have shallow rooting depth relative to perennial grasses (Savelle 1977). Although C was concentrated in the surface 20–40 cm, these data demonstrate significant C storage potential in deeper soil horizons in grasslands dominated by annual plants. Woody plants added significant soil C below 1 m, but annual grasslands without a woody component still showed increasing C content to 1-m depth. This may be due to the presence of residual soil C from the historical presence of woody plants or even perennial grasses, although annual grasses have dominated this region for more than 100 yr. Alternatively it is possible that some grass species may be able to access deeper soils with roots. Some annual grassland plants allocate over 30% of their root mass below 30-cm depth (Gordon and Rice 1992). Soil C may be also translocated down in the soil profile by earthworms or water (Shuster et al. 2001; Mariani et al. 2007). Sequestering C deep in the soil profile could partially offset increasing atmospheric CO_2 concentrations. Soil C stored in subsurface horizons is likely to be less biologically active due to lower resource availability for decomposers, lower redox potential, potentially higher concentrations of more recalcitrant C compounds that resist decay (Silver et al. 1999; Chabbi et al. 2009), and lower probability of soil physical disturbance.

Rangelands with a woody component had significantly more soil C below 10-cm depth than open grasslands. In the top 100 cm this amounted to almost 40 Mg C $\cdot$ ha^{-1}. Our analysis could not account for the species composition, density, biomass, or productivity of woody plants in the rangelands surveyed, nor historical patterns of woody plant distribution and biomass, all of which can impact soil C pools. Regardless, sites that contained woody plants at the time of sampling consistently had high soil C pools, even when controlling for temperature and precipitation. Oak woodlands and wooded savanna make up a significant proportion of the rangelands in California (Griffen 1977). These ecosystems are characterized by tree islands in a grassland matrix. Oak understories tend to have higher soil C and nutrient pools and lower bulk densities than the surrounding grasslands (Dahlgren et al. 1997). Oaks have greater rooting depth than grasses, providing an important contribution to deep soil C. These ecosystems may also be better at retaining C over time due to more complete use of seasonally available water (Ma et al. 2007). Rangeland management, oak removal to increase forage production, increasing urban development, vineyard expansion, and Sudden Oak Death have led to a decline in oaks in California rangelands that may negatively impact C storage and nutrient cycling in these soils (Dahlgren et al. 1997; Giusti et al. 2004; Gaman and Firman 2006; but see Kroodsma and Field 2006).

Oaks are not the only woody plants that occur in California rangelands. Coastal rangelands have been experiencing an invasion of *Baccharis pilularis* over the last 50 yr (McBride and Heady 1968; Hobbs and Mooney 1986; Stromberg et al. 2001; Zavaleta and Kettley 2006). Baccharis invasion increased soil C pools in a semiarid rangeland, but decreased total ANPP by 67% over 25 yr (Zavaletta and Kettley 2006). Woody encroachment is increasing worldwide (Van Auken et al. 2000; Schroter et al. 2005; Hobbs et al. 2006) due to grazing (Schlesinger et al. 1990) and fire management (Bond and Keeley 2005), as well as anthropogenic N deposition (Kochy and Wilson 2001) and elevated atmospheric CO_2 concentrations (Bond and Midgley 2000; Polley et al. 2003). Although woody plants can significantly increase soil C pools, they also present some ecological and economic costs in rangelands (Jackson et al. 2005). Deep-rooting trees can alter the hydrology of grassland ecosystems, increasing salinity and altering nutrient dynamics (Jobbagy and Jackson 2004; McCully et al. 2004; Jackson et al. 2005). Woody plants can decrease forage for livestock, negatively impacting yields. Woody invasion does not always increase soil C pools in grasslands. Jackson et al. (2000) found that woody invasion decreased soil C content in perennial grasslands receiving $\geq$ 660 mm rainfall per year, amounts typical of many Mediterranean climates.

Grazing can impact soil C pools by affecting C inputs via NPP, turnover rates, root to shoot allocation, and C exports via decomposition, erosion, and hydrologic losses. In a review of largely perennial grasslands, Conant et al. (2001) found that improved grazing practices such as moderate stocking rates significantly increased rates of soil C sequestration, averaging 0.35 Mg C $\cdot$ ha^{-1} $\cdot$ yr^{-1}. They found that rates of soil C sequestration were greatest in warm dry regions with high potential evapotranspiration and with a long prior history of grazing, similar to many rangelands in California. In our analysis grazing appeared to have no significant impact on soil C pools in California rangelands. This is not necessarily surprising at the level to which this could be evaluated in this dataset. The timing, frequency, and intensity of grazing, data that were not available for the current analysis, strongly influence a system's response to grazing. For example, the timing of grazing has a strong impact on vegetation composition, particularly the prevalence of later-season, deeper-rooted species (reviewed in Huntsinger et al. 2007; Jackson and Bartolome 2007). The impacts of RDM, an indicator of grazing intensity, on ANPP can vary by site and year (Bartolome et al. 2007), and its impacts on a given site may be masked by analyzing across broadly different conditions. Consideration of such context-dependent interactions is critical for effective ecosystem management (Eviner and Hawkes 2008).

Distribution of Available Data on Soil Carbon Pools

Our review of the literature highlights the range of bioclimatic zones that have been well studied in California and those that are poorly studied. Thirty-nine of the 48 profiles used in our analysis were located at just five sites throughout the state. Obvious gaps in data include annual grasslands in arid regions as well as montane rangelands. More data from these environments will help facilitate modeling at a regional scale. In this study we have focused on soil organic C, but significant inorganic C can occur in some arid and semiarid environments (Eshel et al. 2007) and deserves more attention.

MANAGEMENT IMPLICATIONS

Soil C sequestration has been proposed as a means to help offset greenhouse gas emissions and reduce the atmospheric burden of CO_2 (IPCC 2007). Understanding patterns in soil C storage is a first step to exploring soil C sequestration potential. The wide range in soil C pools in California's rangelands across similar soil types and climate suggests considerable potential to increase soil C storage in these ecosystems through management. The presence of woody plants is likely to facilitate C storage at depth. However, increased woody plant biomass often comes at the expense of forage production, can result in soil salinization, and result in significant water loss at an ecosystem scale (Jobbagy and Jackson 2004; McCully et al. 2004; Jackson et al. 2005). Improved grazing practices, organic amendments, and irrigation have been proposed as mechanisms to increase C storage in rangeland soils. Soil C sequestration in rangelands is likely to be a relatively inexpensive approach to climate change mitigation, with many co-benefits in the form of higher plant production, increased soil water-holding capacity, and decreased soil erosion (Paustian et al. 1997).

LITERATURE CITED

Baisden, W. T., R. Amundson, D. L. Brenner, A. C. Cook, C. Kendall, and J. W. Harden. 2002a. A multiisotope C and N modeling analysis of soil organic matter turnover and transport as a function of soil depth in a California annual grassland soil chronosequence. *Global Biogeochemical Cycles* 16:1135.

Baisden, W. T., R. Amundson, A. C. Cook, and D. L. Brenner. 2002b. Turnover and storage of C and N in five density fractions from California annual grassland surface soils. *Global Biogeochemical Cycles* 16:1117.

Baisden, W. T., and R. L. Parfitt. 2007. Bomb C-14 enrichment indicates decadal C pool in deep soil? *Biogeochemistry* 85:59–68.

Balesdent, J., and M. Balabane. 1996. Major contribution of roots to soil carbon storage inferred from maize cultivated soils. *Soil Biology and Biochemistry* 28:1261–1263.

Bartolome, J. W., R. D. Jackson, A. D. K. Betts, J. M. Connor, G. A. Nader, and K. W. Tate. 2007. Effects of residual dry matter on net primary production and plant functional groups in Californian annual grasslands. *Grass and Forage Science* 62:445–452.

Betts, K. 2003. Demonstrating carbon sequestration. *Environmental Science & Technology* 37:354A–355A.

Bond, W. J., and J. E. Keeley. 2005. Fire as a global 'herbivore': the ecology and evolution of flammable ecosystems. *Trends in Ecology and Evolution* 20:387–394.

Bond, W. J., and G. F. Midgley. 2000. A proposed CO2-controlled mechanism of woody plant invasion in grasslands and savannas. *Global Change Biology* 6:865–869.

Branson, F. A., G. G. Gifford, K. G. Renard, and R. F. Hadley. 1981. Rangeland hydrology. Dubuque, IA, USA: Kendall/Hunt Publishing Company. 84 p.

Bremer, D. J., J. M. Ham, C. E. Owensby, and A. K. Knapp. 1998. Responses of soil respiration to clipping and grazing in a tallgrass prairie. *Journal of Environmental Quality* 27:1539–1548.

Bronson, K. F., T. M. Zobeck, T. T. Chua, V. Acosta-Martinez, R. S. van Pelt, and J. D. Booker. 2004. Carbon and nitrogen pools of southern high plains cropland and grassland soils. *Soil Science Society of America Journal* 68:1695–1704.

Brown, S., A. Dushku, T. Pearson, D. Shoch, J. Winsten, S. Sweet, and J. Kadyszewski. 2004. Carbon supply from changes in management of forest, range, and agricultural lands of California. Sacramento, CA, USA: Winrock International, for the California Energy Commission, PIER Energy-Related Environmental Research. 500-04-068F. 144 p.

Burke, I. C., C. M. Yonker, W. J. Parton, C. V. Cole, K. Flach, and D. S. Schimel. 1989. Texture, climate, and cultivation effects on organic matter content in US grassland soils. *Soil Science Society of America Journal* 53:800–805.

Camping, T. J., R. A. Dahlgren, K. W. Tate, and W. R. Horwath. 2002. Changes in soil quality due to grazing and oak tree removal in California blue oak woodlands. *In:* R. B. Standiford, D. McCreary, and K. L. Purcell (eds.). Oaks in California's changing landscape. Berkeley, CA, USA: US Department of Agriculture, General Technical Report PSW-184. p. 75–85.

Cao, G. M., Y. H. Tang, W. H. Mo, Y. A. Wang, Y. N. Li, and X. Q. Zhao. 2004. Grazing intensity alters soil respiration in an alpine meadow on the Tibetan plateau. *Soil Biology & Biochemistry* 36:237–243.

Carbone, M. S., G. C. Winston, and S. E. Trumbore. 2008. Soil respiration in perennial grass and shrub ecosystems: linking environmental controls with plant and microbial sources on seasonal and diel timescales. *Journal of Geophysical Research* 113:G02022.

Chabbi, A., I. Kogel-Knabner, and C. Rumpel. 2009. Stabilised carbon in subsoil horizons is located in spatially distinct parts of the soil profile. *Soil Biology & Biochemistry* 41:256–261.

Chou, W. W., W. L. Silver, R. D. Jackson, A. W. Thompson, and B. Allen-Diaz. 2008. The sensitivity of annual grassland carbon cycling to the quantity and timing of rainfall. *Global Change Biology* 14:1382–1394.

Christian, J. M., and S. D. Wilson. 1999. Long-term ecosystem impacts of an introduced grass in the northern Great Plains. *Ecology* 80:2397–2407.

Conant, R. T., K. Paustian, and E. T. Elliott. 2001. Grassland management and conversion into grassland: effects on soil carbon. *Ecological Applications* 11:343–355.

Dahlgren, R. A., M. J. Singer, and X. Huang. 1997. Oak tree and grazing impacts on soil properties and nutrients in a California oak woodland. *Biogeochemistry* 39:45–64.

Del Grasso, S. J., D. S. Ojima, W. J. Parton, E. Stehfest, M. Heistemann, B. Deangelo, and S. Rose. 2009. Global scale DAYCENT model analysis of greenhouse gas emissions and mitigation strategies for cropped soils. *Global and Planetary Change* 67:44–50.

Derner, J. D., and G. E. Schuman. 2007. Carbon sequestration and rangelands: a synthesis of land management and precipitation effects. *Journal of Soil and Water Conservation* 62:77–85.

Eshel, G., P. Fine, and M. J. Singer. 2007. Total soil carbon and water quality: an implication for carbon sequestration. *Soil Science Society of America Journal* 71:397–405.

Eviner, V. T., and C. V. Hawkes. 2008. Embracing variability in the application of plant-soil interactions to the restoration of communities and ecosystems. *Restoration Ecology* 16:713–729.

Fierer, N., O. A. Chadwick, and S. E. Trumbore. 2005. Production of CO_2 in soil profiles of a California annual grassland. *Ecosystems* 8:412–429.

Follett, R. F., J. M. Kimble, and R. Lal. 2001. The potential of U.S. grazing lands to sequester carbon and mitigate the greenhouse effect. Boca Raton, FL, USA: Lewis Publishers. 410 p.

Frank, D. A., and S. J. McNaughton. 1993. Evidence for the promotion of aboveground grassland production by native large herbivores in Yellowstone National Park. *Oecologia* 96:157–161.

Gaman, T., and J. Firman. 2006. Oaks 2040: The status and future of oaks in California. Oakland, CA, USA: California Oak Foundation. 55 p.

George, M., J. Bartolome, N. McDougald, M. Connor, C. Vaughn, and G. Markegard. 2001. Annual range forage production. Oakland, CA, USA: Division of Agriculture and Natural Resources, University of California. ANR Publ. 8018. 9 p.

George, M., J. Clawson, J. Menke, and J. Bartolome. 1985. Annual grassland forage productivity. *Rangelands* 7:17–19.

Gessler, P. E., O. A. Chadwick, F. Chamran, L. Althouse, and K. Holmes. 2000. Modeling soil-landscape and ecosystem properties using terrain attributes. *Soil Science Society of America Journal* 64:2046–2056.

Giusti, G. A., R. B. Standiford, D. D. McCreary, A. Merenlender, and T. Scott. 2004. Oak woodland conservation in California's changing landscape. Berkeley, CA, USA: University of California. White paper, IHRMP publication. 6 p.

Gordon, D. R., and K. J. Rice. 1992. Partitioning of space and water between two California annual grassland species. *American Journal of Botany* 79:967–976.

Griffin, J. 1977. Oak woodland. *In:* M. J. Barbour and J. Major (eds.). Terrestrial vegetation of California. New York, NY, USA: John Wiley and Sons. p. 383–415.

Guo, Y. Y., R. Amundson, P. Gong, and Q. Yu. 2006. Quantity and spatial variability of soil carbon in the conterminous United States. *Soil Science Society of America Journal* 70:590–600.

Havstad, K. M., D. C. Peters, B. Allen-Diaz, J. Bartolome, B. T. Bestelmeyer, D. Briske, J. Brown, M. Brunson, J. E. Herrick, L. Huntsinger, P. Johnson, L. Joyce, R. Pieper, A. J. Svejcar, and J. Yao. 2009. The western United States rangelands, a major resource. *In:* W. F. Wedin and S. L. Fales (eds.). Grassland quietness and strength for a new American agriculture. Madison, WI, USA: American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America. p. 75–93.

Heady, H., J. Bartolome, M. Pitt, G. Savelle, and M. Stroud. 1991. California prairie. *In:* R. T. Coupland (ed.). Natural grasslands. Ecosystems of the world. Volume 8A. Amsterdam, the Netherlands: Elsevier Scientific. p. 313–335.

Heady, H. F. 1956. Changes in a California annual plant community induced by manipulation of natural mulch. *Ecology* 37:798–812.

Heady, H. F. 1965. The influence of mulch on herbage production in an annual grassland. *Proceedings of the 9th International Grassland Congress* 1:391–394.

Hedrick, D. W. 1948. The mulch layer of California annual ranges. *Journal of Rangeland Management* 1:22–25.

Herman, D. J., L. J. Halverson, and M. K. Firestone. 2003. Nitrogen dynamics in an annual grassland: oak canopy, climate, and microbial population effects. *Ecological Applications* 13:593–604.

Hobbs, R. J., S. Arico, J. Aronson, J. S. Baron, P. Bridgewater, V. A. Cramer, P. R. Epstein, J. J. Ewel, C. A. Klink, A. E. Lugo, D. Norton, D. Ojima, D. M. Richardson, E. W. Sanderson, F. Valladares, M. Vila, R. Zamora, and M. Zobel. 2006. Novel ecosystems: theoretical and management aspects of the new ecological world order. *Global Ecology and Biogeography* 15:1–7.

Hobbs, R. J., and H. A. Mooney. 1986. Community changes following shrub invasion of grassland. *Oecologia* 70:508–513.

Holland, E. A., W. J. Parton, J. K. Detling, and D. L. Coppock. 1992. Physiological responses of plant populations to herbivory and their consequences for ecosystem nutrient flow. *American Naturalist* 140:685–706.

Holland, V. L., and D. J. Kiel. 1995. California vegetation. Dubuque, IA, USA: Kendall/Hunt Publishing Company. 516 p.

Huntsinger, L., J. W. Bartolome, and C. M. D'Antonio. 2007. Grazing management of California grasslands. *In:* J. Corbin, M. Stromberg, and C. M. D'Antonio (eds.). Berkeley, CA, USA: University of California Press. p. 233–297.

[IPCC] Intergovernmental Panel on Climate Change. 2007. Climate change 2007: synthesis report. Contribution of Working Groups I, II and III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Core Writing Team, R. K. Pachauri and A. Reisinger [eds.]. Geneva, Switzerland: IPCC. 104 p.

Jackson, L. E., R. B. Strauss, M. K. Firestone, and J. W. Bartolome. 1988. Plant and soil nitrogen dynamics in California annual grassland. *Plant and Soil* 110:9–17.

Jackson, R. B., J. Canadell, J. R. Ehleringer, H. A. Mooney, O. E. Sala, and E. D. Schulze. 1996. A global analysis of root distributions for terrestrial biomes. *Oecologia* 108:389–411.

Jackson, R. B., E. G. Jobbagy, R. Avissar, S. B. Roy, D. J. Barrett, C. W. Cook, K. A. Farley, D. C. le Maitre, B. A. McCarl, and B. C. Murray. 2005. Trading water for carbon with biological sequestration. *Science* 310:1944–1947.

Jackson, R. B., H. J. Schenk, E. G. Jobbagy, J. Canadell, G. D. Colello, R. E. Dickinson, C. B. Field, P. Friedlingstein, M. Heimann, K. Hibbard, D. W. Kicklighter, A. Kleidon, R. P. Neilson, W. J. Parton, O. E. Sala, and M. T. Sykes. 2000. Belowground consequences of vegetation change and their treatment in models. *Ecological Applications* 10:470–483.

Jackson, R. D., and J. W. Bartolome. 2002. A state-transition approach to understanding nonequilibrium plant community dynamics in Californian grasslands. *Plant Ecology* 162:49–65.

Jackson, R. D., and J. W. Bartolome. 2007. Grazing ecology of California grasslands. *In:* J. Corbin, M. Stromberg, and C. M. D'Antonio (eds.). Ecology and management of California grasslands. Berkeley, CA, USA: University of California Press. p. 197–206.

Jobbagy, E. G., and R. B. Jackson. 2000. The vertical distribution of soil organic carbon and its relation to climate and vegetation. *Ecological Applications* 10:423–436.

Jobbagy, E. G., and R. B. Jackson. 2004. The uplift of soil nutrients by plants: biogeochemical consequences across scales. *Ecology* 85:2380–2389.

Johnson, L. C., and J. R. Matchett. 2001. Fire and grazing regulate belowground processes in tallgrass prairie. *Ecology* 82:3377–3389.

Kieft, T. L. 1994. Grazing and plant-canopy effects on semiarid soil microbial biomass and respiration. *Biology and Fertility of Soils* 18:155–162.

Kochy, M., and S. D. Wilson. 2001. Nitrogen deposition and forest expansion in the northern Great Plains. *Journal of Ecology* 89:807–817.

Kroodsma, D. A., and C. B. Field. 2006. Carbon sequestration in Califronia agriculture, 1980–2000. *Ecological Applications* 16:1975–1985.

Lal, R. 2004. Agricultural activities and the global carbon cycle. *Nutrient Cycling in Agroecosystems* 70:103–116.

Lal, R., J. Kimble, E. Levine, and B. A. Stewart (eds.). 1995.Soil management and greenhouse effect. Boca Raton, FL, USA: Lewis Publishers. 385 p.

Lehmann, J. 2007. Bio-energy in the black. *Frontiers in Ecology and the Environment* 5:381–387.

Loeser, M. R., T. E. Crews, and T. D. Sisk. 2004. Defoliation increased above-ground productivity in a semi-arid grassland. *Journal of Range Management* 57:442–447.

Ma, F. S., S. Z. Kang, F. S. Li, J. H. Zhang, T. S. Du, X. T. Hu, and M. X. Wang. 2007a. Effect of water deficit in different growth stages on stem sap flux of greenhouse grown pear-jujube tree. *Agricultural Water Management* 90:190–196.

Masiello, C. A., O. A. Chadwick, J. Southon, M. S. Torn, and J. W. Harden. 2004. Weathering controls on mechanisms of carbon storage in grassland soils. *Global Biogeochemical Cycles*. 18:GB4023, doi: 10.1029/2004GB002219.

Mariani, L., J. J. Jimenez, J. N. Asakawa, R. J. Thomas, and T. Decaens. 2007. What happens to earthworm casts in the soil? A field study of carbon and nitrogen dynamics in neotropical savannahs. *Soil Biology and Biochemistry* 39:757–767.

McBride, J., and H. F. Heady. 1968. Invasion of grassland by *Baccharis pilularis. Journal of Range Management* 21:106–108.

McCulley, R. L., E. G. Jobbagy, W. T. Pockman, and R. B. Jackson. 2004. Nutrient uptake as a contributing explanation for deep rooting in arid and semi-arid ecosystems. *Oecologia* 141:620–628.

McNaughton, S. J. 1985. Ecology of a grazing ecosystem—the Serengeti. *Ecological Monographs* 55:259–294.

Miller, R. M., and J. D. Jastrow. 1990. Hierarchy of root and mycorrhizal fungal interactions with soil aggregation. *Soil Biology & Biochemistry* 22:579–584.

Parton, W. J., D. S. Ojima, and D. S. Schimel. 1994. Environmental change in grasslands—assessment using models. *Climatic Change* 28:111–141.

Paustian, K., E. Levine, W. M. Post, and I. M. Ryzhova. 1997. The use of models to integrate information and understanding of soil C at the regional scale. *Geoderma* 79:227–260.

Polley, H. W., H. B. Johnson, and C. R. Tischler. 2003. Woody invasion of grasslands: evidence that CO2 enrichment indirectly promotes establishment of Prosopis glandulosa. *Plant Ecology* 164:85–94.

Post, W. M., and K. C. Kwon. 2000. Soil carbon sequestration and land-use change: processes and potential. *Global Change Biology* 6:317–327.

Potter, K. N., and J. D. Derner. 2006. Soil carbon pools in central Texas: prairies, restored grasslands, and croplands. *Journal of Soil and Water Conservation* 61:124–128.

Puget, P., and L. E. Drinkwater. 2001. Short-term dynamics of root- and shoot-derived carbon from a leguminous green manure. *Soil Science Society of America Journal* 65:771–779.

Sanderman, J., and R. Amundson. 2008. A comparative study of dissolved organic carbon transport and stabilization in California forest and grassland soils. *Biogeochemistry* 89:309–327.

Sanderman, J., J. A. Baldock, and R. Amundson. 2008. Dissolved organic carbon chemistry and dynamics in contrasting forest and grassland soils. *Biogeochemistry* 89:181–198.

Savelle, G. D. 1977. Comparative structure and function in a California annual and native bunchgrass community [dissertation]. Berkeley, CA, USA: University of California.

Schimel, D. S., B. H. Braswell, E. A. Holland, R. McKeown, D. S. Ojima, T. H. Painter, W. J. Parton, and A. R. Townsend. 1994. Climatic, edaphic, and biotic controls over storage and turnover of carbon in soils. *Global Biogeochemical Cycles* 8:279–293.

Schlesinger, W. H., J. F. Reynolds, G. L. Cunningham, L. F. Huenneke, W. M. Jarrell, R. A. Virginia, and W. G. Whitford. 1990. Biological feedbacks in global desertification. *Science* 247:1043–1048.

Schroter, D., W. Cramer, R. Leemans, I. C. Prentice, M. B. Araújo, N. W. Arnell, A. Bondeau, H. Bugmann, T. R. Carter, C. A. Graciah, A. de la Vega-Leinert, M. Erhard, F. Ewert, M. Glendining, J. I. House, S. Kankaanpaa, R. J. T. Klein, S. Lavorel, M. Lindner, M. J. Metzger, J. Meyer, T. D. Mitchell, I. Reginster, M. Rounsevell, S. Sabate, S. Sitch, B. Smith, J. Smith, P. Smith, M. T. Sykes, K. Thonicke, W. Thuiller, G. Tuck, S. Zaehle, and B. Zierl. 2005. Ecosystem service supply and vulnerability to global change in Europe. *Science* 310:1333–1337.

Schuman, G. E., H. H. Janzen, and J. E. Herrick. 2002. Soil carbon dynamics and potential carbon sequestration by rangelands. *Environmental Pollution* 116:391–396.

Shuster, W. D., S. Subler, and E. L. McCoy. 2001. Deep-burrowing earthworm additions changed the distribution of soil organic carbon in a chisel-tilled soil. *Soil Biology and Biochemistry* 33:983–996.

Silver, W. L., A. E. Lugo, and D. Farmer. 2002. Soil organic carbon in tropical forests of the U. S. *In:* J. Kimble, R. Birdsey, L. Heath, R. Follett, and R. Ratan (eds.). The potential of U.S. forest soils to sequester carbon and mitigate the greenhouse effect. Boca Raton, FL, USA: Lewis Publishers. p. 363–382.

Silver, W. L., A. E. Lugo, and M. Keller. 1999. Soil oxygen availability and biogeochemistry along rainfall and topographic gradients in upland wet tropical forest soils. *Biogeochemistry* 44:301–328.

Silver, W. L., R. Ostertag, and A. E. Lugo. 2000. The potential for carbon sequestration through reforestation of abandoned tropical agricultural and pasture lands. *Restoration Ecology* 8:394–407.

Six, J. A., E. T. Elliott, and K. Paustian. 2000. Soil macro aggregate turnover and micro aggregate formation: a mechanism for C sequestration under no-tillage agriculture. *Soil Biology and Biochemistry* 32:2099–2103.

Smith, P. 2004. Soils as carbon sinks: the global context. *Soil Use and Management* 20:212–218.

Smith, P., C. M. Fang, J. J. C. Dawson, and J. B. Moncrieff. 2008. Impact of global warming on soil organic carbon. *Advances in Agronomy* 97:1–43.

Soussana, J. F., P. Loiseau, N. Vuichard, E. Ceschia, J. Balesdent, T. Chevallier, and D. Arrouays. 2004. Carbon cycling and sequestration opportunities in temperate grasslands. *Soil Use and Management* 20:219–230.

Steenwerth, K. L., L. E. Jackson, F. J. Calderon, M. R. Stromberg, and K. M. Scow. 2002. Soil microbial community composition and land use history in cultivated and grassland ecosystems of coastal California. *Soil Biology and Biogeochemistry* 34:1599–1611.

Stromberg, M. R., P. Kephart, and V. Yadon. 2001. Composition, invasibility, and diversity in coastal California grasslands. *Madrono* 48:236–252.

Trumbore, S. E., O. A. Chadwick, and R. Amundson. 1996. Rapid exchange between soil carbon and atmospheric carbon dioxide driven by temperature change. *Science* 272:393–396.

Turner, C. L., T. R. Seastedt, and M. I. Dyer. 1993. Maximization of aboveground grassland production—the role of defoliation frequency, intensity, and history. *Ecological Applications* 3:175–186.

Van Auken, O. W. 2000. Shrub invasions of North American semiarid grasslands. *Annual Review of Ecology and Systematics* 31:197–215.

Waldrop, M. P., and M. K. Firestone. 2006. Seasonal dynamics of microbial community composition and function in oak canopy and open grassland soils. *Microbial Ecology* 52:470–479.

Zavaleta, E. S., and L. S. Kettley. 2006. Ecosystem change along a woody invasion chronosequence in a California grassland. *Journal of Arid Environments* 66:290–306.

Ecological Applications, 22(2), 2012, pp. 634–647

New effects of Roundup on amphibians: Predators reduce herbicide mortality; herbicides induce antipredator morphology

Rick A. Relyea[1]

Department of Biological Sciences, University of Pittsburgh, Pittsburgh, Pennsylvania 15260 USA

Abstract. The use of pesticides is important for growing crops and protecting human health by reducing the prevalence of targeted pest species. However, less attention is given to the potential unintended effects on nontarget species, including taxonomic groups that are of current conservation concern. One issue raised in recent years is the potential for pesticides to become more lethal in the presence of predatory cues, a phenomenon observed thus far only in the laboratory. A second issue is whether pesticides can induce unintended trait changes in nontarget species, particularly trait changes that might mimic adaptive responses to natural environmental stressors. Using outdoor mesocosms, I created simple wetland communities containing leaf litter, algae, zooplankton, and three species of tadpoles (wood frogs [*Rana sylvatica* or *Lithobates sylvaticus*], leopard frogs [*R. pipiens* or *L. pipiens*], and American toads [*Bufo americanus* or *Anaxyrus americanus*]). I exposed the communities to a factorial combination of environmentally relevant herbicide concentrations (0, 1, 2, or 3 mg acid equivalents [a.e.]/L of Roundup Original MAX) crossed with three predator-cue treatments (no predators, adult newts [*Notophthalmus viridescens*], or larval dragonflies [*Anax junius*]). Without predator cues, mortality rates from Roundup were consistent with past studies. Combined with cues from the most risky predator (i.e., dragonflies), Roundup became less lethal (in direct contrast to past laboratory studies). This reduction in mortality was likely caused by the herbicide stratifying in the water column and predator cues scaring the tadpoles down to the benthos where herbicide concentrations were lower. Even more striking was the discovery that Roundup induced morphological changes in the tadpoles. In wood frog and leopard frog tadpoles, Roundup induced relatively deeper tails in the same direction and of the same magnitude as the adaptive changes induced by dragonfly cues. To my knowledge, this is the first study to show that a pesticide can induce morphological changes in a vertebrate. Moreover, the data suggest that the herbicide might be activating the tadpoles' developmental pathways used for antipredator responses. Collectively, these discoveries suggest that the world's most widely applied herbicide may have much further-reaching effects on nontarget species than previous considered.

Key words: American toads (Bufo americanus *or* Anaxyrus americanus); *amphibian decline; dragonflies* (Anax junius); *glyphosphate; inducible defense; leopard frogs* (Rana pipiens *or* Lithobates pipiens); *newts* (Notophthalmus viridescens); *phenotypic plasticity; synergy; wood frogs* (Rana sylvatica *or* Lithobates sylvaticus).

Introduction

To understand the impacts of anthropogenic chemicals on natural communities, we often base predictions on the plethora of single-species tests that are conducted as part of the pesticide regulation process. While a valuable first-step in determining the potential lethality of contaminants in nature over short periods (i.e., 1–4 d), these tests may tell us little about the impacts of contaminants on organisms under more natural conditions and over longer periods (Relyea and Hoverman 2006, Clements and Rohr 2009). Thus, many ecologists and toxicologists have moved to examine the impacts of contaminants under more natural conditions and over more realistic exposure times. In this manner, we can gain a better understanding of the direct and indirect effects of contaminants on nontarget organisms when embedded within their natural community.

Natural communities contain a number of potential factors that might interact with contaminants. Stressors, broadly defined as environmental factors that impair individual performance, can act either additively or synergistically. For contaminants, a number of abiotic and biotic stressors can make contaminants more or less lethal (Relyea and Hoverman 2006). Among biotic stressors, stress from low food or high competition can increase the lethality of contaminants (Postma et al. 1994, Hanazato and Hirokawa 2004, Beketov and Liess 2005, Jones et al. 2011). In addition, stress from the presence of predators can make several pesticides more lethal (Relyea and Mills 2001, Relyea 2003*b*, 2004*c*,

Manuscript received 2 February 2011; revised 2 May 2011; accepted 9 August 2011; final version received 9 September 2011. Corresponding Editor (ad hoc): D. W. Sparling.

[1] E-mail: relyea@pitt.edu

2005*d*). However, synergistic interactions between pesticides and predatory stress have only been observed under laboratory conditions. We need to determine whether this lethal synergy occurs under more natural conditions.

While experiments have primarily examined whether natural stressors affect the lethality of contaminants, it is equally important to examine whether contaminants affect an organism's ability to adaptively respond to natural stressors. Any synergistic or antagonistic interactions of contaminants with stressors could cause organisms to become poorly suited to their environment. For example, biotic stressors such as competition and predation induce changes in the behavior, physiology, morphology, and life history of individuals (Miner et al. 2005). We have a good understanding of how contaminants alter the behavioral traits of target and nontarget animals, especially in the case of insecticides that commonly act on the animal's nervous system (reviewed in Weis et al. 2001). However, we know considerably less about the effects of contaminants on morphological traits, including those that are induced as a result of environmental stressors. For example, contaminants could interfere with an individual's normal environmental induction of morphology and cause the individual to develop a morphological phenotype that is poorly suited to the current environment. Such effects could alter morphological traits in ways that increase or decrease an individual's performance and ultimately its fitness. In some species of zooplankton, for example, insecticides can either induce morphological changes typically associated with predator defenses (Hanazato 1991, Barry 1998, 1999) or inhibit the induction of such traits (Hanazato 1999, Barry 1999, 2000). Beyond a few species of zooplankton, we have little information on the ability of contaminants to impact the induced morphological defenses of other taxonomic groups (but see Teplitsky et al. 2005).

I addressed these challenges using a mesocosm experiment in which I exposed an assemblage of three tadpole species to three levels of predatory stress, each in the presence of four concentrations of a globally common herbicide (glyphosate; commercially sold under many names including Roundup and Vision; Monsanto Corporation, St. Louis, Missouri, USA). Commercial formulations of glyphosate can be moderately to highly toxic to tadpoles under environmentally relevant concentrations (Bernal et al. 2009, Relyea and Jones 2009). Moreover, under laboratory conditions, the herbicide can be more lethal to tadpoles in the presence of waterborne cues emitted by predators (Relyea 2005*d*). To my knowledge, it is unknown whether predators can make the herbicide more lethal under mesocosm conditions nor whether the herbicide can induce morphological changes in any animal species. Tadpoles are an excellent model system in this regard because they are well known for expressing predator-induced changes in morphology including the development of relatively deeper tail fins (Van Buskirk 2002, Relyea 2003*b*, 2004*a*).

Methods

Pesticide Background

Roundup Original MAX is one of numerous glyphosate-based herbicide formulations sold around the world by a variety of manufacturers. Collectively, glyphosate-based herbicides are the number one herbicide in the world with sales growing rapidly with the marketing of Roundup-Ready crops (Baylis 2000). Glyphosate products are used by homeowners, industry, and agriculture to kill undesirable plants. Glyphosate kills plants by preventing them from producing essential amino acids. For most plants, glyphosate alone has difficulty penetrating plant tissues due to the presence of the leaf cuticle layer, so a surfactant is typically added to introduce glyphosate into the plant. Polyethoxylated tallow amine (POEA) is one of the most commonly used surfactants. This surfactant can be highly toxic to fish and amphibians at application rates that are found in nature (Relyea 2006). While the surfactant of Roundup Original MAX is a trade secret (S. Mortenson, *personal communication*), the formulation has a toxicity to amphibians that is nearly identical to those formulations that are known to contain POEA (Relyea 2005*d*, Relyea and Jones 2009).

The concentrations of glyphosate-based herbicides in wetlands depend on whether the applications are inadvertent (e.g., applications over forests; Thompson et al. 2004) or due to drift, soil run-off, and plant wash-off. Expected worst-case concentrations, based on a range of assumptions regarding application rates, water depth, and interception by vegetation, range from 1.4 to 7.6 mg a.e./L (where a.e. stands for acid equivalents; Boutin et al. 1995, Mann and Bidwell 1999, Giesy et al. 2000, Solomon and Thompson 2003). Observed worst-case concentrations range from 1.7 to 5.2 mg a.e./L (Edwards et al. 1980, Giesy et al. 2000, Thompson et al. 2004). The half-life of glyphosate in pond water ranges from 8 to 120 d depending on environmental conditions (Barolo 1993).

The mesocosm experiment

The experiment employed a completely randomized design containing a factorial combination of four nominal Roundup concentrations (0, 1, 2, or 3 mg a.e./L of glyphosate) crossed with three predator treatments (no predator, caged adult newts [*Notophthalmus viridescens*], and caged larval dragonflies [*Anax junius*]). The resulting 12 treatment combinations were replicated four times for a total of 48 experimental units.

The experimental units were 757-L, outdoor mesocosms that contained many components of natural wetlands. The plastic mesocosms were filled with approximately 570 L of well water on 18–20 April 2006. On 21 April, I added 200 g of leaf litter (*Quercus* spp.) and 15 g of rabbit chow; both items serve as initial nutrient sources and the leaf litter also serves as a prey refuge. On the same day, I collected pond water from five ponds that contained zooplankton, phytoplankton, and periphyton. After screening the water for predators, I mixed water from all the ponds and added an aliquot of the resulting slurry to each tank. Soil was not added to the mesocosms because previous research has found that adding soil has no effect on the toxicity of Roundup under mesocosm conditions (Relyea 2005*c*). Each tank was covered with a 65% shade cloth lid to prevent other organisms from colonizing the tanks. Each tank also was equipped with four plastic lids (area = 45 cm^2) attached to a length of weighted plastic pipe and oriented vertically to serve as periphyton samplers.

Algal and zooplankton communities developed for 20 d, after which I added three species of larval amphibians. Amphibians were collected as newly oviposited eggs (wood frogs, *Rana sylvatica* [*Lithobates sylvaticus*] = 15 egg masses; northern leopard frogs, *R. pipiens* [*L. pipiens*] = 10 egg masses, American toads, *Bufo americanus* [*Anaxyrus americanus*] = 10 egg masses). The eggs were hatched and raised in wading pools containing aged well water and fed rabbit chow ad libitum. On 10 May (defined as day 0), I added 30 tadpoles of each species to every mesocosm for a total density of 90 tadpoles per mesocosm. This density of tadpoles (22 per m^2) is within the range of natural densities for these species during early ontogeny (R.A. Relyea, *unpublished data*). The tadpoles were early in development (approximately Gosner stage 25; Gosner 1960) with the following initial mean masses (±SE): wood frogs, 58 ± 4 mg; leopard frogs, 36 ± 1 mg; American toads, 16 ± 1 mg.

The predator treatments were added on day 1. Each mesocosm was equipped with two predator cages constructed from 236-mL plastic cups covered with a screen held on with a rubber band. In mesocosms assigned the no-predator treatment, the cages remained empty. In mesocosms assigned the newt or dragonfly treatments, each cage contained a single predator that was fed approximately 300 mg of tadpoles (a mixture of wood frogs and leopard frogs) three times per week. On each feeding day, I also lifted the empty cages in the no-predator treatment to equalize disturbance across all tanks.

The herbicide treatments were applied on day 2. I used a popular formulation of glyphosate (Roundup Original MAX; Monsanto Corporation, St. Louis, Missouri, USA) that contained 540 mg a.e./L of glyphosate plus an undisclosed surfactant. To attain the nominal concentrations of 0, 1, 2, and 3 mg a.e./L of glyphosate, I added 0, 0.864, 1.728, and 2.592 mg of formulated product to the mesocosms, respectively. The formulated product was added to 300 mL well water and then this mixture was distributed evenly across the surface of the mesocosms. One hour after the applications, I sampled the water of all mesocosms in the middle of the water column and pooled the samples by herbicide treatment. The water samples were then frozen and later shipped to the Mississippi State Chemical Lab (Mississippi State, Mississippi, USA) for analysis using high-pressure liquid chromatography. The analyses indicated that the actual concentrations were 0.9, 1.8, and 3.4 mg a.e./L. Given that these values are within 13% of the nominal values with no directional bias, the concentrations will hereafter be referred to as 1, 2, and 3 mg a.e./L.

On the afternoon of day 9, I measured temperature, pH, and dissolved oxygen of all mesocosms. Measurements were taken with a calibrated, digital water meter. Across all treatments, there was little variation in pH (range, 7.8–8.0) or temperature (range, 11.7–11.9°C). In contrast, dissolved oxygen varied widely among treatments (range, 10–20 mg/L), so I analyzed the treatment effects on dissolved oxygen.

On days 15 and 21, I sampled the periphyton in each mesocosm. On each date, I removed two periphyton sampler discs from the same location in each mesocosm, brushed them free of periphyton, and then rinsed the samplers with well water. The slurry of periphyton and water was filtered through pre-dried and pre-weighed Whatman GF/C filters. After filtering the periphyton slurry, the filters were dried for 24 h at 80°C and re-weighed to estimate periphyton availability in each mesocosm.

On day 21, I terminated the experiment by removing all water and leaf litter and recovering all surviving tadpoles. The survivors were euthanized in 2% MS-222 and then preserved in 10% formalin. The preserved animals were later counted and weighed to determine survival and average individual mass for each species. The amphibian response variables were the proportion of each species surviving in each mesocosm and the mean individual mass of each species in each mesocosm.

Morphological measurements

One of the objectives of this study was to examine whether exposure to the herbicide affected tadpole morphology. Because a large proportion of tadpoles died in the highest herbicide treatment, I excluded this treatment from the morphological analyses. Two of the three species (wood frogs and leopard frogs) possess highly plastic morphological responses to predators and competitors (Relyea and Werner 1999, Relyea 2003*a*, Schoeppner and Relyea 2009), so only these two species were assessed for predator- and herbicide-induced changes in relative morphology. Past studies on American toads suggests that they are less plastic (Relyea 2001). I measured seven morphological traits on each surviving tadpole: tail depth and length, body depth, length, and width, and tail muscle depth and width (see Fig. 1 in Relyea 2000).

Statistical analyses

I analyzed the data using analyses of variance. I conducted separate multivariate analyses of variance

TABLE 1. Multivariate and univariate test results examining the effects of three predator cue treatments crossed with four concentrations of glyphosphate (as the commercial formulation Roundup Original MAX) on the survival of wood frog, leopard frog, and American toad tadpoles.

	Multivariate test (Wilks' lambda)			Univariate tests (*P*)			
Treatment	df	*F*	*P*	df	Wood frog survival	Leopard frog survival	Toad survival
Herbicide	9,108	7.2	<0.001	3,36	<0.001	<0.001	<0.001
Predator	6,70	2.6	0.027	2,36	0.047	0.077	0.068
Herbicide × Predator	18,108	1.6	0.063	6,36	0.010	0.068	0.618

Note: In the univariate tests, only df and *P* values are reported.

(MANOVAs) on survival and mass, because some mesocosms at the highest glyphosate concentration had no surviving animals to permit an assessment of mass. The survival data for wood frogs and leopard frogs were heteroscedastic and the data for leopard frogs marginally non-normal. The data distribution could not be improved by any transformation, but analyses of variance are robust to such violations when the data are balanced and independent (Quinn and Keough 2002), particularly when the largest variance is not more than four times larger than the smallest variance (which was the case for leopard frog survival but not for wood frog survival). The mass data were both homoscedastic and normally distributed. The analysis of mass did not include survival as a covariate because there was no indication that treatments that caused tadpole mortality were associated with increased individual mass due to reduced competition.

To estimate the LC50 values (i.e., the concentration required to kill 50% of a population) for each species under each predator treatment, I conducted probit analyses. Using 84% confidence intervals for these estimates allows one to determine differences among predator treatments because non-overlapping 84% confidence intervals are significant at approximately $\alpha = 0.05$ (Payton et al. 2003).

The next set of analyses examined the effects of the treatments on tadpole morphology. Because linear morphological measurements are typically larger on animals of larger mass, I examined mass-adjusted morphological traits. To do this, I conducted a MANCOVA on all seven log-transformed linear dimensions and included log-transformed mass as a covariate and the two treatments (predators and pesticides) as fixed effects. For most traits in both species, I was able to confirm that there were no significant two- or three-way mass-by-treatment interactions (a key assumption in mass-adjustment analyses). However, there was a significant multivariate mass-by-predator interaction in wood frogs. Examination of the univariate responses indicated that there was an interaction of mass and predators effect on tail depth; whereas the smallest animals had tail depths of similar size, the magnitude of predator induction on tail depth continually increased as tadpole mass increased. This is not surprising given that the ability to express a plastic response in morphology increases with increased growth (Relyea 2004*b*). Ignoring this mass-by-predator interaction would estimate the magnitude of predator induction for a tadpole of average mass. This overestimates predator induction of the smallest tadpoles and underestimates predator induction of the largest tadpoles, but provides an unbiased estimate of predator induction for average sized tadpoles. In contrast, there were no significant mass-by-herbicide interactions on tadpole morphology, so any herbicide induction of tadpole morphology could be estimated without bias.

From the MANCOVA, I saved the residuals from each tadpole and the estimated marginal means for each treatment. I added these two values to determine the mass-adjusted morphology of each tadpole. For wood frog and leopard tadpoles, I then calculated mesocosm means, which served as response variables.

The mass-adjusted morphological traits of the leopard frogs and wood frogs were analyzed using separate MANOVAs. The leopard frog data had homoscedastic errors and were normally distributed. The wood frog data had homoscedastic errors for all of the morphological traits except for muscle width and all data were normally distributed. Significant multivariate effects were followed by univariate tests and pairwise comparisons (Tukey's hsd).

I also analyzed treatment effects on periphyton biomass and dissolved oxygen concentrations. Periphyton biomass was analyzed using a repeated-measures analyses of variance (rm-ANOVA). The log-transformed data were normally distributed and the errors were either homoscedastic or marginally homoscedastic. Dissolved oxygen data were analyzed using an ANOVA. Assumptions of normality and homoscedasticity were both met. All mean comparisons were conducted using Tukey's hsd.

RESULTS

Tadpole survival

There were multivariate effects of herbicide and predators and a marginal concentration-by-predator interaction (Table 1, Fig. 1). The subsequent ANOVAs illuminated which species were driving these effects.

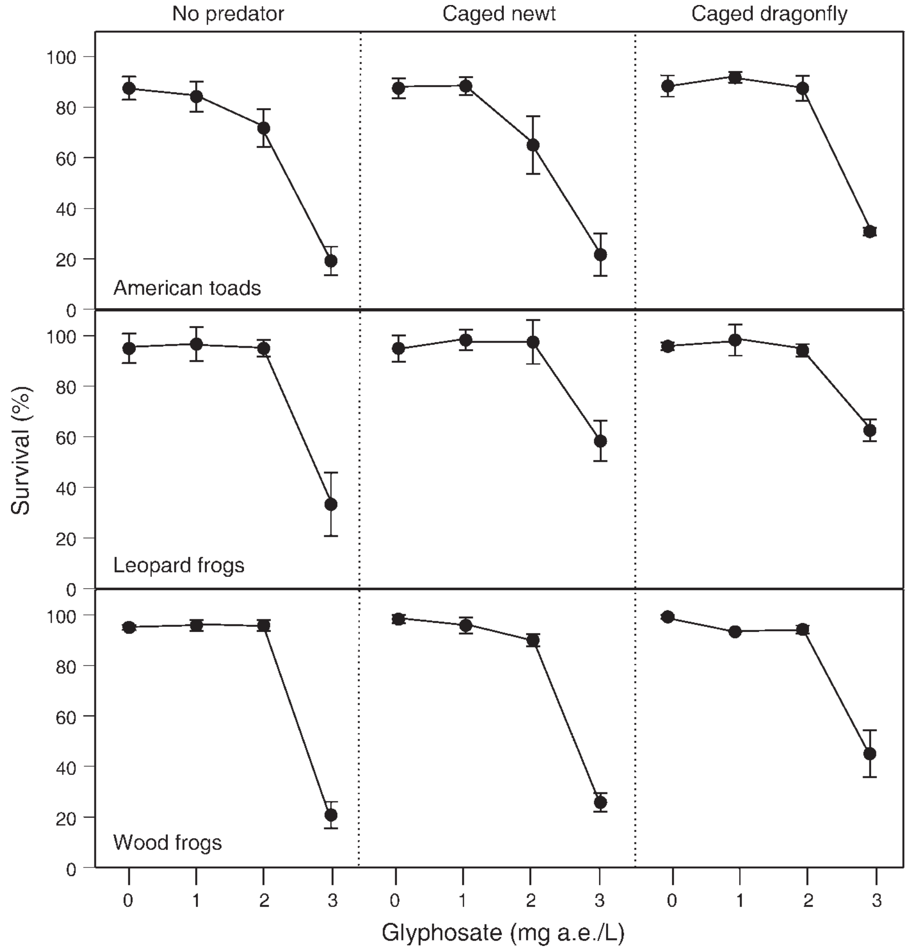


FIG. 1. The survival of three species of tadpoles when exposed to a factorial combination of caged-predator treatments crossed with four concentrations of glyphosphate (as the commercial formulation Roundup Original MAX). Data are means ± SE; "a.e." stands for acid equivalents.

American toad survival was affected by the herbicide and there was a marginal effect of predators, but no herbicide-by-predator interaction (Table 1, Fig.1). Toads in the controls averaged 88% survival and toads exposed to 1 mg a.e./L exhibited no reduction in survival ($P = 0.955$). In contrast, toads exposed to 2 mg a.e./L experienced an average survival of 75% ($P = 0.011$) and toads exposed to 3 mg a.e./L experienced an average survival of 24% ($P < 0.001$). Although the herbicide-by-predator interaction was not significant, it is clear from the data that the marginally significant predator effect was driven by glyphosate's lethal effects being reduced in the presence of caged dragonflies. For example, at 2 mg a.e./L, survival was reduced by 16% with no predators but was only reduced by 1% with caged dragonflies. Similarly, at 3 mg a.e./L, survival was reduced by 68% with no predators but was only reduced by 58% with caged dragonflies. Based on the probit analyses, the LC50 for toads ranged from 2.4 to 2.8 mg a.e./L (Table 2).

TABLE 2. Estimated LC50 values (i.e., the concentration required to kill 50% of a population) for three species of tadpoles when exposed to a range of concentrations of glyphosphate (as the commercial formulation Roundup Original MAX) in the presence of three caged-predator environments.

Species	Caged predator	LC50	84% CI
Wood frog	no predator	2.95	-
	caged newt	2.63	2.45, 2.84
	caged dragonfly	3.09	2.75, 3.80
Leopard frog	no predator	2.91	2.74, 3.03
	caged newt	3.02	-
	caged dragonfly	3.26	3.02, 3.90
American toad	no predator	2.46	2.27, 2.69
	caged newt	2.44	2.26, 2.64
	caged dragonfly	2.82	2.66, 2.96

Notes: Estimates are followed by 84% confidence intervals; nonoverlapping confidence intervals are significant at approximately $\alpha = 0.05$ (Payton et al. 2003). In two cases, indicated by dashes, the confidence interval could not be estimated due to the distribution of the data.

Leopard frog survival was affected by the herbicide and marginally affected by predators and the concentration-by-predator interaction (Table 1, Fig.1). Once again, the interaction occurred because the impact of the herbicide was larger without predators than with caged dragonflies. Increasing the herbicide from 0 to 3 mg a.e./L caused 62% death without predators, but only 37% death with caged newts and 33% death with caged dragonflies (all $P \leq 0.002$). Based on the probit analyses, the LC50 ranged from 2.9 to 3.2 mg a.e./L (Table 2).

Wood frog survival was affected by herbicide, predator, and an herbicide-by-predator interaction (Table 1, Fig.1). The interaction occurred because increasing the herbicide concentration from 0 to 3 mg a.e./L caused 74% death without predators and 73% death with caged newts, but caused only 54% death with caged dragonflies (all $P < 0.001$). Based on the probit analyses, the LC50 for wood frogs ranged from 2.6 to 3.1 mg a.e./L (Table 2).

Tadpole mass

Tadpole mass exhibited significant effects of herbicide, predators, and the herbicide-by-predator interaction (Table 3, Fig. 2). Subsequent ANOVAs determined which of the three tadpole species was driving these multivariate effects. American toad mass exhibited no effects of glyphosate or predators (Table 3, Fig. 2).

Leopard frog mass exhibited a main effect of herbicide, no main effect of predators, and a significant interaction (Table 3, Fig. 2). Without predators, there was no effect of the herbicide ($P > 0.3$). With caged newts, there also was no effect of the herbicide ($P > 0.08$). With caged dragonflies, however, there was no effect of 1 or 2 mg a.e./L ($P > 0.7$) compared to the control, but there was a large (37%) decline in mass with 3 mg a.e./L ($P = 0.001$).

Wood frog mass was affected by herbicide, predators, and their interaction (Table 3, Fig. 2). To understand the nature of the interaction, I examined how increased herbicide concentrations affected growth compared to the control within each predator treatment. When predators were absent, there was no effect of 1 mg a.e./L ($P = 0.434$) compared to the control, a marginal decline in mass (13%) with 2 mg a.e./L ($P = 0.070$), and a significant decline in mass (15%) with 3 mg a.e./L ($P = 0.032$). With caged newts, there was no effect of 1 or 2 mg a.e./L ($P > 0.3$) compared to the control, but there was a marginal decline in mass (17%) with 3 mg a.e./L ($P = 0.054$). With caged dragonflies, there was no effect of 1 or 2 mg a.e./L ($P > 0.9$) compared to the control, but there was a large (38%) decline in mass with 3 mg a.e./L ($P < 0.001$).

Tadpole morphology

Leopard frogs.—The analysis of leopard frog morphology found significant multivariate effects of the herbicide and predator treatments, but no herbicide-by-predator interaction (Fig. 3, Table 4). Univariate analyses indicated that these effects were caused by herbicide and predator effects on tail depth, body depth, and body length (Table 4).

Tail depth was affected by both the predator and herbicide treatments (Table 4). Compared to the no-predator control, caged newts induced no change ($P = 0.176$) while caged dragonflies induced deeper tails ($P = 0.004$). Compared to the herbicide control, 1 mg a.e./L had no effect on tail depth ($P = 0.984$) while 2 mg a.e./L induced deeper tails ($P = 0.006$).

Body depth was affected by the herbicide treatments but not by the predator treatments (Table 4). There was no difference between 0 mg a.e./L and the other two concentrations (both $P > 0.2$), but bodies were deeper with 2 mg a.e./L than with 1 mg a.e./L ($P = 0.014$).

Body length was affected by the predator treatments but not by the herbicide treatments (Table 4). Compared to the no-predator treatment, newts induced similar body lengths ($P = 0.677$) while dragonflies induced shorter body lengths ($P = 0.044$).

In summary, caged dragonflies induced leopard frogs to develop deeper tails and shorter bodies than the no-predator control. Interestingly, 2 mg a.e./L of the herbicide also induced deeper tails as well as changes in body depth.

Wood frogs.—The analysis of wood frog morphology found significant multivariate effects of the herbicide and predator treatments, but no herbicide-by-predator interaction (Fig. 3, Table 5). Univariate tests indicated that the treatments affected four morphological traits: muscle depth, tail depth, body depth, and body length.

The muscle depth of wood frogs was only affected by the herbicide treatments (Table 5). Compared to 0 mg a.e./L, 1 mg a.e./L had no effect ($P = 0.933$) but 2 mg a.e./L induced deeper tail muscles ($P = 0.009$).

Wood frog tail depth was affected by both the predator and the herbicide treatments (Table 5). Compared to the no-predator control, caged newts had no effect ($P = 0.123$) and caged dragonflies induced significantly deeper tails ($P < 0.001$). Compared to 0 mg a.e./L, 1 mg a.e./L had no effect ($P = 0.990$) but 2 mg a.e./L induced deeper tails ($P = 0.011$).

Body depth was marginally affected by the herbicide but not by predators (Table 5). Bodies exposed to 0 mg a.e./L tended to be deeper than 1 mg a.e./L ($P = 0.057$) but not different from 2 mg a.e./L ($P = 0.661$). The latter two concentrations did not differ ($P = 0.288$).

Body length was affected by the predator treatments but not by the herbicide treatments (Table 5). Compared to the no-predator control, body length was marginally reduced by caged newts ($P = 0.081$) and significantly reduced by caged dragonflies ($P = 0.005$).

In summary, caged dragonflies induced wood frogs to develop deeper tails and shorter bodies than the no-predator control. An exposure to 2 mg a.e./L of the herbicide induced deeper tails and tail muscles than 0 mg a.e./L. The herbicide also induced changes in body depth.

TABLE 3. Multivariate and univariate test results examining the effects of three predator cue treatments crossed with four concentrations of glyphosphate (as the commercial formulation Roundup Original MAX) on the mass of wood frog, leopard frog, and American toad tadpoles.

Treatment	Multivariate test (Wilks' lambda) df	F	P	Univariate tests (P) df	Wood frog mass	Leopard frog mass	Toad mass
Herbicide	9,105	4.4	<0.001	2,27	<0.001	0.002	0.508
Predator	6,68	2.5	0.030	2,27	0.002	0.146	0.622
Herbicide × Predator	18,105	2.5	0.002	4,27	0.002	0.014	0.117

Note: In the univariate tests, only df and *P* values are reported.

Periphyton and dissolved oxygen

The analysis of periphyton found an effect of herbicide ($F_{3,34} = 4.3$, $P = 0.012$) and time ($F_{1,34} = 18.3$, $P < 0.001$), but no predator effect ($F_{2,34} = 0.05$, $P = 0.952$) or any interactions (all $P > 0.17$; Fig. 4). Periphyton declined over time, but averaged over time and across all predator treatments, periphyton was similarly abundant between the control and either 1 or 2 mg a.e./L ($P > 0.9$), but was 83% more abundant with 3 mg a.e./L ($P = 0.039$).

The ANOVA on dissolved oxygen found a significant effect of herbicide ($F_{3,36} = 14.4$, $P < 0.001$), but no effect of predator ($F_{2,36} = 0.2$, $P = 0.797$) or an herbicide-by-predator interaction ($F_{6,36} = 0.6$, $P = 0.707$; Fig. 4). Averaged across predators, dissolved oxygen was highest in the absence of the herbicide. Compared to

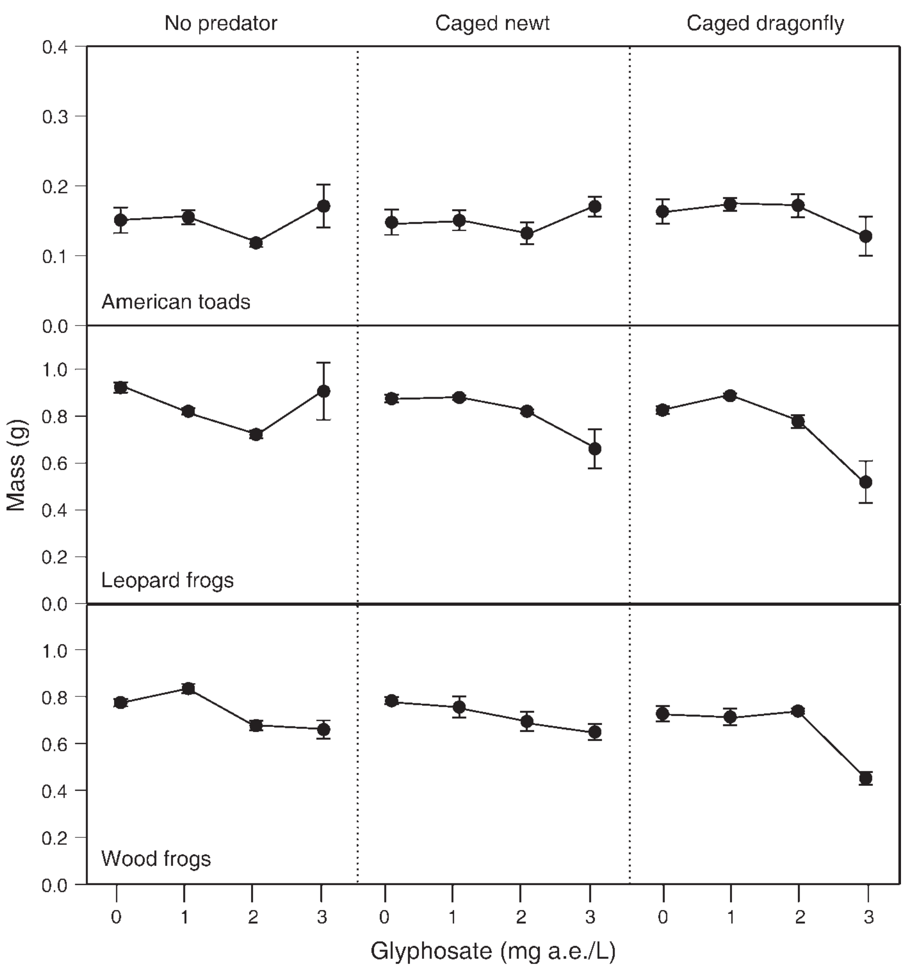


FIG. 2. The mass of three species of tadpoles when exposed to a factorial combination of caged-predator treatments crossed with three concentrations of glyphosphate (as the commercial formulation Roundup Original MAX). The highest nominal concentration used in the experiment (3 mg a.e./L) resulted in too few survivors to reliably assess tadpole mass. Data are means ± SE.

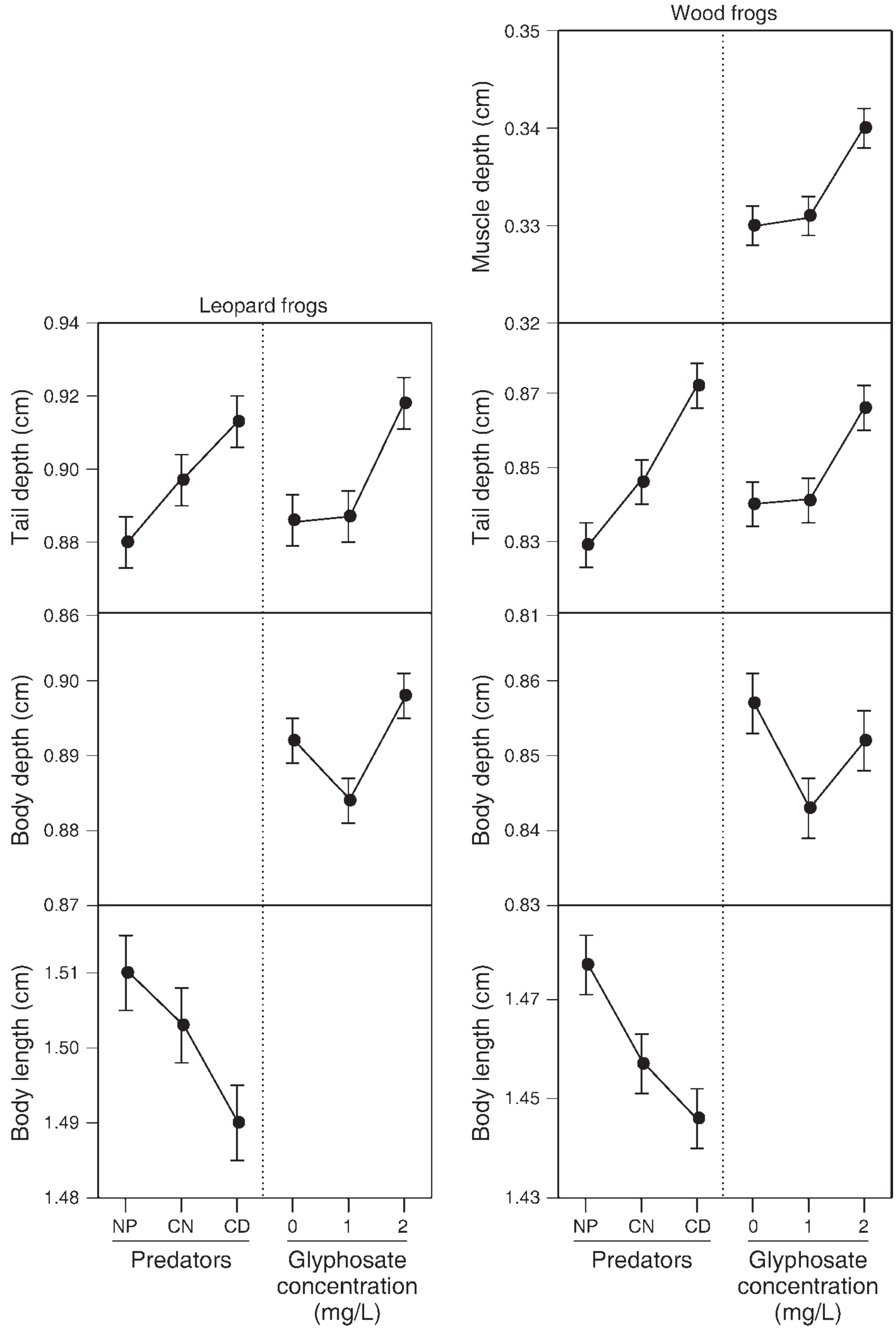


FIG. 3. The relative morphology of leopard frog and wood frog tadpoles when exposed to a factorial combination of caged-predator treatments crossed with three concentrations of glyphosphate (as the commercial formulation Roundup Original MAX). Because the predator and herbicide treatments did not interact, the displayed predator effects are averaged across herbicide treatments, and the displayed herbicide effects are averaged predator treatments. The highest nominal concentration used in the experiment (3 mg a.e./L) resulted in too few survivors to assess morphology. Data are means ± SE. Only significant main effects are shown.

the control, additions of 1, 2, or 3 mg a.e./L caused 28 to 35% reductions in dissolved oxygen (all $P < 0.001$). The latter three treatments did not differ from each other (all $P > 0.6$). Across all herbicide concentrations, dissolved oxygen concentrations remained quite high.

DISCUSSION

The experiment demonstrated that glyphosate-based herbicides and predator cues can have surprising effects on amphibians and wetland communities. Environmentally relevant concentrations of the herbicide caused

TABLE 4. Multivariate and univariate test results examining the effects of three predator cue treatments crossed with three concentrations of glyphosphate (as the commercial formulation Roundup Original MAX) on the relative morphology of leopard frog tadpoles.

	Multivariate test (Wilks' lambda)			Univariate tests (P)							
					Tail		Body			Muscle	
Treatment	df	F	P	df	Depth	Length	Depth	Length	Width	Depth	Width
Herbicide	14,42	2.9	0.003	2,27	0.003	0.807	0.019	0.329	0.457	0.224	0.441
Predator	14,42	2.3	0.021	2,27	0.006	0.863	0.986	0.050	0.996	0.826	0.113
Herbicide × Predator	28,77	1.3	0.169								

Note: In the univariate tests, only df and P values are reported.

high rates of mortality in three species of tadpoles, reductions in growth for two of the three species (i.e., wood frogs and leopard frogs), and several indirect effects including an increase in periphyton and a decrease in dissolved oxygen (although oxygen concentrations were always well above those that would cause any harmful effects). When the different concentrations were crossed with the chemical cues emitted by predators, the herbicide exposures became less lethal which is in direct contrast to lab studies that have found that predator cues can make the herbicide more lethal. As in past studies, the tadpoles responded to the highest level of predatory stress (i.e., caged dragonflies) by altering their morphology in adaptive directions. Unexpectedly, however, the herbicide also induced changes in the tadpole's morphology with a direction and magnitude that were nearly identical to the morphological changes induced by the caged dragonflies. To my knowledge, this is the first example of pesticide-induced morphological plasticity in amphibians or any other vertebrate.

Predators facilitate tadpole survival when exposed to herbicides

The mortality caused by the herbicide was within the range observed in past experiments. Across the three species and the three predator environments, LC50 values ranged from 2.4 to 3.3 mg a.e./L. A large number of laboratory studies have been conducted on commercial formulations of the herbicide containing either the POEA surfactant or an undisclosed surfactant possessing a toxicity similar to POEA (e.g., Roundup Original, Roundup Original MAX, Roundup Weathermax, Vision, Cosmo-Flux). These lab studies, which typically change the water and reapply the pesticide every 1–4 d, have found LC50 values ranging from 0.4 to 11.6 mg a.e./L (Mann and Bidwell 1999, Lajmanovich et al. 2003, Edginton et al. 2004, Howe et al. 2004, Relyea 2005*d*, Bernal et al. 2009, Relyea and Jones 2009, Dinehart et al. 2010). Based on the standard toxicity definitions used by the U.S. Environmental Protection Agency, these commercial formulations range from slightly toxic (10 mg/L < LC50 < 100 mg/L) to highly toxic (0.1 mg/L < LC50 < 1 mg/L; toxicity definitions *available online*).[2]

A growing number of toxicity studies have been conducted under mesocosm conditions and have observed effects that are consistent with the laboratory studies. For example, Relyea (2005*b*) found that 3 mg a.e./L caused high rates of tadpole mortality, with several species including wood frogs, leopard frogs, American toads, and gray tree frogs (*Hyla versicolor*) being nearly or completely eliminated. A follow-up study tested whether the addition of sand or loam soil would ameliorate the effects of Roundup on three species of tadpoles (leopard frogs, American toads, and gray tree frogs). In that study, neither sand nor loam reduced the high rates of tadpole mortality (Relyea 2005*c*). Using one-third as much Roundup (1 mg a.e./L), Relyea et al. (2005) found no effect on gray tree frog tadpoles, but observed 29% death in leopard frogs and 71% in American toads. As in the current experiment, all of these mesocosm experiments were conducted using well water that had an approximate pH = 8. More recently, Jones et al. (2010) observed similar high mortality levels in wood frog and American toad tadpoles exposed to Roundup concentrations up to 3 mg a.e./L, but found that applications later in ontogeny were less lethal than applications earlier in ontogeny. Using a different suite of species (American bullfrogs [*R. catesbeiana*], green frogs [*R. clamitans*], and gray tree frogs), Jones et al. (2011) found that the high rates of mortality at 2 and 3 mg a.e./L can be further increased under conditions of increased competition. Importantly, experiments conducted at lower pH values have found lower rates of mortality at similar concentrations of glyphosate + POEA (Wojtaszek et al. 2004). As noted by past authors, this means that the toxicity of these herbicide formulations are of particular concern in wetlands on the upper end of the naturally occurring pH range (Chen et al. 2004, Edginton et al. 2004).

As reviewed by Relyea (2011), a key issue in evaluating the potential impact of glyphosate-based herbicides is to consider the concentrations found in nature. Unfortunately, there is a paucity of data on the

[2] http://www.epa.gov/espp/litstatus/effects/redleg-frog/

TABLE 5. Multivariate and univariate test results examining the effects of three predator cue treatments crossed with three concentrations of glyphosphate (as the commercial formulation Roundup Original MAX) on the relative morphology of wood frog tadpoles.

	Multivariate test (Wilks' lambda)			Univariate tests (*P*)							
					Tail		Body			Muscle	
Treatment	df	*F*	*P*	df	Depth	Length	Depth	Length	Width	Depth	Width
Herbicide	14,42	2.8	0.005	2,27	0.005	0.178	0.066	0.288	0.650	0.006	0.903
Predator	14,42	3.1	0.002	2,27	<0.001	0.949	0.323	0.006	0.802	0.326	0.255
Herbicide × Predator	28,77	1.0	0.463								

Note: In the univariate tests, only df and *P* values are reported.

concentrations of glyphosate in natural ponds and wetlands because most major surveys of pesticides in water bodies have been restricted to lakes, streams, and rivers. In contrast, most amphibians do not live in these habitats. For ponds and wetlands the expected worst-case scenarios for terrestrial formulations of glyphosate (which are generally not recommended for aquatic applications) range from 1.4 to 7.6 mg a.e./L depending on the assumptions used (Boutin et al. 1995, Mann and Bidwell 1999, Giesy et al. 2000, Solomon and Thompson 2003). Actual worst-case scenarios range from 1.7 to 5.2 mg a.e./L (Edwards et al. 1980, Giesy et al. 2000, Thompson et al. 2004). Mean concentrations across a landscape of inadvertently oversprayed wetlands can be considerably lower e.g., 0.33 mg a.e./L; Thompson et al. 2004). However, it is important to realize that a given pond does not experience the mean concentration, but instead receives a specific concentration. For example, while Thompson et al. (2004) found that the mean concentration in forested wetlands (after spraying to favor conifer trees over broadleaf trees) was 0.33 mg a.e./L, they found that individual wetlands had up to 1.95 mg a.e./L, a concentration that can cause high amphibian mortality.

While the high toxicity of this globally common herbicide has been well documented under both lab and mesocosm conditions, the observation that predator cues can make the herbicide less lethal under mesocosm conditions is a novel discovery. The most likely explanation for this observation is herbicide stratification. Recent studies have discovered that the water column can experience thermal stratification which, in turn, causes glyphosate-based herbicides to be two to four times more concentrated near the surface than near the benthos (Jones et al. 2010, 2011). Although the current experiment sampled the water in the middle of the water column rather than separately sampling near the surface and near the benthos, it is reasonable to assume that the mesocosms in the current study also experienced stratification, given that the current study and Jones et al. (2010) were conducted in the same location, during similar times of year, under similar conditions, and using the same formulation of Roundup. Because the stratification of lentic habitats is a common phenomenon, it is not surprising in hindsight that pesticides can stratify. Indeed, pesticide stratification is a phenomenon that has been observed in natural wetlands in at least two studies (Sudo et al. 2004, Ma et al. 2008). The frequency of such stratification will depend on ambient temperatures, pond depth, and the existence of winds that can potentially cause a stratified water column to mix. What is interesting is that the stratification of Roundup sets the stage for the interactive effects of predator cues and herbicide concentrations.

The current study included three predator treatments that represented increasing levels of predation risk to tadpoles (Babbitt 2001, Relyea 2003*a*) that typically induce different magnitudes of antipredator responses. When predator cages are positioned at the surface,

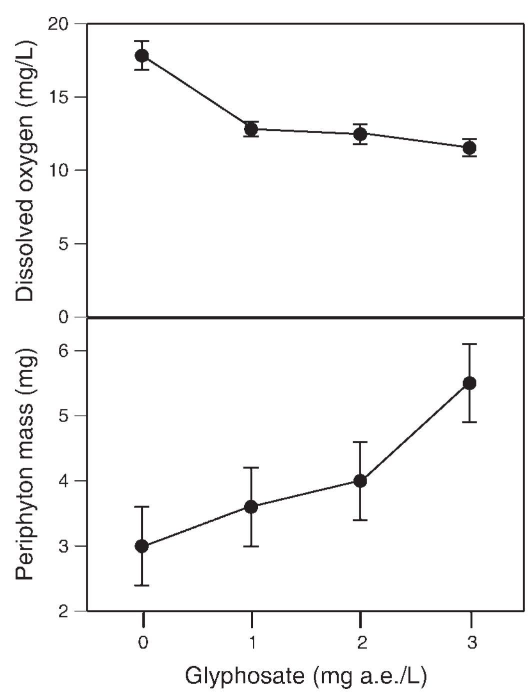


FIG. 4. The biomass of periphyton (averaged across samples taken on day 15 and 21) and the dissolved concentration of oxygen (on day 9) in mesocosms exposed to a factorial combination of caged-predator treatments crossed with four concentrations of glyphosphate (as the commercial formulation Roundup Original MAX). Periphyton was sampled on two dates. Samples are based on two 45-cm^2 samplers per mesocosm on each date. Data are means ± SE.

tadpoles typically move down to the benthos (Relyea 2001, Schoeppner and Relyea 2009) and this behavior was noted in the current study (R. A. Relyea, *personal observation*). This move to the benthos would have placed the tadpoles in a region of the water column that had a lower herbicide concentration and, as a result, fewer tadpoles died. In short, herbicide stratification and predator-induced changes in the habitat use of tadpoles combined to reduce the amount of herbicide-caused mortality by 20% in wood frogs, 29% in leopard frogs, and 10% in toads. The smaller effect in toads might be due to the caged predators being fed only tadpoles of wood frogs and leopard frogs; past studies have shown that tadpoles exhibit small differences in their antipredator traits when predators consume tadpole diets that span different families (Schoeppner and Relyea 2005). Given that predator avoidance is common in a wide range of aquatic taxa (Lima 2002), it is possible that predator cues and the stratification of contaminants could cause interactive effects in other taxonomic groups as well.

To my knowledge, the only experimental assessment of predatory stress and glyphosate-based herbicides was the lab experiment of Relyea (2005*d*) which tested six species of tadpoles in 8-L tubs of water and found that one of the species (wood frogs) experienced significantly *higher* rates of mortality when the herbicide was combined with predatory cues emitted by caged newts (in that study, leopard frogs and American toads exhibited no synergistic interactions). In the current experiment, the synergy was observed for wood frogs and leopards (significant for wood frogs, marginally nonsignificant for leopard frogs), but in the *opposite* direction. However, the density of caged newts in the lab experiments (one newt in 8 L of water) was much higher than the density of caged newts in the current mesocosm experiment (four newts in 570 L of water). Although each newt in the mesocosm experiment was also fed three times more tadpole biomass per day than the lab experiments, the concentration of predator cues in the earlier lab experiments was still nearly six times more concentrated than the current mesocosm experiment. Assuming increased concentrations of predator cue lead to stronger synergistic interactions with pesticides, this difference should make it less likely that predator cues would increase the lethality of the herbicide in the mesocosm experiment. Importantly, however, this difference in predator cue concentration does not explain the observation that predator cues actually *decreased* the lethality of the herbicide in the mesocosm experiment.

Tadpole mass and periphyton abundance

Changes in tadpole survival were concomitant with herbicide and predator effects on tadpole mass in two of the three species (i.e., wood frogs and leopard frogs). In general, the strongest reductions in tadpole mass occurred when tadpoles were exposed to the most risky predator (i.e., dragonflies) combined with the highest concentration of the herbicide. Because the periphyton was more abundant under the highest herbicide concentration, due to fewer surviving tadpoles, the reduced growth of the tadpoles was not due to the herbicide inhibiting periphyton growth. Indeed, under lab conditions in which tadpoles were fed a constant per-capita food ration, many species of tadpoles grew slower when exposed to 1.5 mg a.e./L of glyphosate + POEA (Relyea 2004*b*). Under laboratory conditions, therefore, it seems that the herbicide can affect the ability of tadpoles to consume their food or convert their food into growth. Under mesocosm conditions, however, a reduction in tadpole growth has not been previously observed. For example, herbicide applications of 1 to 3 mg a.e./L (similar to the no-predator treatments in the current experiment) early in tadpole development had no effect on tadpole growth (Jones et al. 2010). In another experiment that manipulated herbicide concentration and tadpole competition, Jones et al. (2011) found that tadpole growth actually increased with higher herbicide concentrations. The key observation in the current experiment is that the decline in tadpole mass only occurred when cues from the most dangerous predator (which induces reduced foraging activity; Relyea 2002*b*, 2003*a*) were combined with the highest concentration of the pesticide. In short, it appears that a reduction in tadpole mass requires both stressors to be present. Reductions in mass are important to amphibians because reduced mass at metamorphosis is associated with reduced post-metamorphic survival, longer times to reproductive maturity, reduced size at maturity, reduced mating success, and smaller clutches of eggs (Smith 1987, Semlitsch et al. 1988, Altwegg and Reyer 2003).

The sharp decline in tadpole survival caused by the highest concentrations of Roundup was the most likely cause of the increased standing crop of periphyton (i.e., the food source of tadpoles). If the herbicide had negative direct effects on periphyton, they were more than overcome by the indirect positive effects of removing the tadpole grazers. Such an outcome would be expected whenever consumers are food limited, as in the current experiment in which a total of 90 tadpoles were added to each mesocosm. A similar increase in periphyton was observed in a previous mesocosm experiment that was initiated with a high density of periphyton consumers (i.e., 50 tadpoles and 30 snails). In that experiment, adding 3 mg a.e./L of Roundup caused high rates of tadpole mortality and a concomitant increase in periphyton (Relyea 2005*b*). Similarly, an experiment using 60–140 tadpoles per mesocosm found that adding 1–3 mg a.e./L of the herbicide caused an increase in periphyton biomass (Jones et al. 2011). In contrast, a mesocosm experiment that was initiated with 40 tadpoles found no effects on periphyton biomass (Jones et al. 2010). Collectively, these studies suggest that herbicide-caused increases in periphyton are more likely when there is more intense competition among tadpoles.

Given that higher concentrations of the herbicide were associated with increases in periphyton, it is perhaps surprising that there was a decline in the concentration of dissolved oxygen for all three herbicide additions (although dissolved oxygen never approached a level that would negatively impact the tadpoles). Increased microbial decomposition of dead tadpoles in the 3 mg a.e./L compared to 1 mg a.e./L treatment does not explain this pattern because dissolved oxygen was similar in these two treatments. Instead, it seems more likely that the herbicide may have been inhibiting the growth of the phytoplankton in the water column. Indeed, past studies have shown that Roundup can cause declines in phytoplankton (Perez et al. 2007), with LC50 values ranging from 1.9 to 5.8 mg a.e./L (Tsui and Chi 2003). Because phytoplankton was not measured in this experiment, the hypothesized mechanism should be examined in future work.

The induction of tadpole morphology

Leopard frogs and wood frogs exhibited changes in relative morphology when exposed to predators and the herbicide. Predator-induced morphology is taxonomically widespread and appears to be an adaptive response to reduce the tadpole's risk of being killed by predators (Van Buskirk 2002, Relyea 2003*a*, 2004*a*, 2005*a*). In most cases, tadpoles respond to predators by building a relatively deep tail fin, which helps evade deadly predator strikes, at the cost of building a relatively smaller body, which leads to slower growth likely due to reduced food consumption and reduced digestive efficiency (Relyea 2000, Relyea and Auld 2004, 2005). As in the case of behavioral responses, tadpoles typically exhibit stronger morphological responses to the most risky predators. Not surprisingly, the current study found that both wood frog and leopard frog tadpoles exhibited weak morphological induction in response to the less risky newts but strong morphological induction in response to dragonflies.

Surprisingly, the herbicide-induced changes in tadpole morphology and did so in the same direction and with the same magnitude as the caged dragonflies. The change in tail depth was particularly striking; for leopard frogs and wood frogs, respectively, the addition of dragonfly chemical cues caused a 3.8% and 5.2% increase in tail depth and the addition of Roundup induced a 3.6% and 3.1% increase in tail depth. It is also interesting that the two factors had additive effects on tail depth, rather than synergistic or antagonistic effects. The combination of dragonfly cues and the herbicide (at 2 mg a.e./L) induced a 6.7–9.3% increase in tail depth for leopard frogs and wood frogs, respectively; this was approximately twice as large as either factor induced alone. The fitness consequences of herbicide-induced plasticity is unknown. However, based on past studies of predator-induced morphology, one would predict that this herbicide induction would have an associated cost of slower growth (Relyea 2002*a*), but this was not observed within the 0 to 2 mg a.e./L range of concentrations that caused morphological induction. Although the mechanism underlying the ability of Roundup to induce morphological changes in tadpoles is unknown, it is a reasonable hypothesis that the herbicide may be interfering with the stress hormones that induce antipredator defenses (Glennemeier and Denver 2002).

Examples of a pesticide inducing morphological changes in animals are rare and limited to a few species of cladocerans. Nearly two decades ago, Hanazato (1991) reported that several organophosphate and carbamate insecticides induced morphological changes in *Daphnia ambigua* that resembled the same morphological changes induced by predators. Two more recent studies have found insecticides can induce elongated crests, a predator-induced phenotype, in *Daphnia* (Barry 1998, Oda et al. 2011). On the other hand, studies on *Bosmina fatalis* have found that insecticides can impede the induction of predator-induced morphology (Barry 1999, 2000, Sakamoto et al. 2006). There appear to be no studies examining the effects of herbicides on the defensive morphology of cladocerans.

Conclusions

The results of this study demonstrate that the impact of the most widely applied herbicide in the world has a number of unexpected effects that often interact with the common natural stressor of predator cues. The high rates of mortality demonstrated in previous studies at environmentally relevant concentrations were fully supported in the current study, further confirming that glyphosate-based herbicides containing the POEA surfactant (or similar surfactants) have the potential to kill large numbers of larval amphibians. The interactive effects of predators and the herbicide are best explained by the previously established stratification of the herbicide. It is reasonable to expect that many other pesticides can also stratify in lentic habitats. Perhaps the most striking discovery was that the herbicide was capable of inducing changes in tadpole morphology in a direction and magnitude that appeared to mimic the adaptive morphological changes induced by predators. Future work should investigate the generality of this phenomenon across all amphibians and across similar types of pesticides. Future studies should also determine the underlying mechanisms of herbicide-induced morphology to determine if herbicides and predator cues activate shared endocrinological pathways.

Acknowledgments

My thanks to Josh Auld, Nicole Diecks, Jason Hoverman, Devin Jones, and Aaron Stoler for their assistance with the experiment. I also thank Kayla Yurco for her excellent attention to detail when measuring the thousands of tadpoles for this study and John Hammond for running the LC50 probit analyses. The manuscript was improved by the comments of Aaron Stoler and Rickey Cothran. This study was funded by the National Science Foundation.

Literature Cited

Altwegg, R., and H.-U. Reyer. 2003. Patterns of natural selection on size at metamorphosis in water frogs. Evolution 57:872–882.

Babbitt, K. J. 2001. Behaviour and growth of southern leopard frog (*Rana sphenocephala*) tadpoles: effects of food and predation risk. Canadian Journal of Zoology 79:809–814.

Barolo, D. 1993. Reregistration eligibility decision for glyphosate. EPA 738-R- 93-014. Reregistration Report. U.S. Environmental Protection Agency, Washington, D.C., USA.

Barry, M. J. 1998. Endosulfan-enhanced crest induction in *Daphnia longicephala*: Evidence for cholinergic innervation of kairomone receptors. Journal of Plankton Research 20:1219–1231.

Barry, M. J. 1999. The effects of a pesticide on inducible phenotypic plasticity in *Daphnia*. Environmental Pollution 104:217–224.

Barry, M. J. 2000. Effects of endosulfan on *Chaoborus*-induced life-history shifts and morphological defenses in *Daphnia pulex*. Journal of Plankton Research 22:1705–1718.

Baylis, A. D. 2000. Why glyphosate is a global herbicide: strengths, weaknesses and prospects. Pesticide Management Science 56:299–308.

Beketov, M. A., and M. Liess. 2005. Acute contamination with esfenvalerate and food limitation: chronic effects on the mayfly, *Cleon dipterum*. Environmental Toxicology and Chemistry 24:1281–1286.

Bernal, M. H., K. R. Solomon, and G. Carrasquilla. 2009. Toxicity of formulated glyphosate (Glyphos) and Cosmo-Flux to larval Colombian frogs 1. Laboratory acute toxicity. Journal of Toxicology and Environmental Health, Part A 72:961–965.

Boutin, C., K. E. Freemark, and C. J. Keddy. 1995. Overview and rationale for developing regulatory guidelines for nontarget plant testing with chemical pesticides. Environmental Toxicology and Chemistry 14:1465–1475.

Chen, C. Y., K. M. Hathaway, and C. L. Folt. 2004. Multiple stress effects of Vision® herbicide, pH, and food on zooplankton and larval amphibian species from forest wetlands. Environmental Toxicology and Chemistry 23:823–831.

Clements, W. H., and J. R. Rohr. 2009. Community responses to contaminants: using basic ecological principles to predict ecotoxicological effects. Environmental Toxicology and Chemistry 28:1789–1800.

Dinehart, S. K., L. M. Smith, S. T. McMurry, P. N. Smith, T. A. Anderson, and D. A. Haukos. 2010. Acute and chronic toxicity of Roundup Weathermax® and Ignite® 280SL to larval *Spea multiplicata* and *S. bombifrons* from the Southern High Plains, USA. Environmental Pollution 158:2610–2617.

Edginton, A. N., P. M. Sheridan, G. R. Stephenson, D. G. Thompson, and H. J. Boermans. 2004. Comparative effects of pH and Vision® herbicide on two life stages of four anuran amphibian species. Environmental Toxicology and Chemistry 23:815–822.

Edwards, W. M., G. B. Triplett, Jr., and R. M. Kramer. 1980. A watershed study of glyphosate transport in runoff. Journal of Environmental Quality 9:661–665.

Giesy, J. P., S. Dobson, and K. R. Solomon. 2000. Ecotoxicological risk assessment for Roundup® herbicide. Reviews of Environmental Contamination and Toxicology 167:35–120.

Glennemeier, K. A., and R. J. Denver. 2002. Small changes in whole-body corticosterone content affect larval *Rana pipiens* fitness components. General and Comparative Endocrinology 127:16–25.

Gosner, K. L. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. Herpetologica 16:183–190.

Hanazato, T. 1991. Pesticides as chemical agents inducing helmet formation in *Daphnia ambigua*. Freshwater Biology 26:419–424.

Hanazato, T. 1999. Anthropogenic chemicals (insecticides) disturb natural organic chemical communication in the plankton community. Environmental Pollution 105:137–142.

Hanazato, T., and H. Hirokawa. 2004. Changes in vulnerability of Daphnia to an insecticide application depending on the population phase. Freshwater Biology 49:402–409.

Howe, C. M., M. Berrill, P. D. Pauli, C. C. Helbring, K. Werry, and N. Veldhoen. 2004. Toxicity of glyphosate-cased pesticides to four North American frog species. Environmental Toxicology and Chemistry 23:1928–1938.

Jones, D. K., J. R. Hammond, and R. A. Relyea. 2010. Roundup® and amphibians: the importance of concentration, application time, and wetland stratification. Environmental Toxicology and Chemistry 29:2016–2025.

Jones, D. K., J. R. Hammond, and R. A. Relyea. 2011. Roundup® and amphibians: Competitive stress can make the herbicide Roundup® more deadly to larval amphibians. Environmental Toxicology and Chemistry 30:446–454.

Lajmanovich, R. C., M. T. Sandoval, and P. M. Peltzer. 2003. Induction of mortality and malformation in *Scinax nasicus* tadpoles exposed to glyphosate formulations. Bulletin of Environmental Contamination and Toxicology 70:612–618.

Lima, S. L. 2002. Putting predators back into behavioral predator-prey interactions. Trends in Ecology and Evolution 17:70–75.

Ma, S., S. C. Kassinos, D. F. Kassinos, and E. Akylas. 2008. Modeling the impact of water withdrawal schemes on the transport of pesticides in the Kouris Dam (Cyprus). Global NEST Journal 10:350–358.

Mann, R. M., and J. R. Bidwell. 1999. The toxicity of glyphosate and several glyphosate formulations to four species of southwestern Australian frogs. Archives of Environmental Contamination and Toxicology 36:193–199.

Miner, B. G., S. E. Sultan, S. G. Morgan, D. K. Padilla, and R. A. Relyea. 2005. Ecological consequences of phenotypic plasticity. Trends in Ecology and Evolution 20:687–692.

Oda, S., Y. Kato, H. Watanabe, N. Tatarazako, and T. Iguchiz. 2011. Morphological changes in *Daphnia galeata* induce by a crustacean terpenoid hormone and its analog. Environmental Toxicology and Chemistry 30:232–238.

Payton, M. E., M. H. Greenstone, and N. Schenker. 2003. Overlapping confidence intervals or standard error intervals: what do they mean in terms of statistical significance? Journal of Insect Science 3:34.

Perez, G. L., et al. 2007. Effects of the herbicide Roundup on freshwater microbial communities: a mesocosm study. Ecological Applications 17:2310–2322.

Postma, J. F., M. C. Buckert-de Jogn, N. Staats, and C. Davids. 1994. Chronic toxicity of cadmium to *Chironomus riparius* (Diptera: Chironomidae) at different food levels. Archives of Environmental Contamination and Toxicology 26:143–148.

Quinn, G. P., and M. J. Keough. 2002. Experimental design and data analysis for biologists. Cambridge University Press, Cambridge, UK.

Relyea, R. A. 2000. Trait-mediated effects in larval anurans: Reversing competition with the threat of predation. Ecology 81:2278–2289.

Relyea, R. A. 2001. Morphological and behavioral plasticity of larval anurans in response to different predators. Ecology 82:523–540.

Relyea, R. A. 2002*a*. Competitor-induced plasticity in tadpoles: consequences, cues, and connections to predator-induced plasticity. Ecological Monographs 72:523–540.

Relyea, R. A. 2002*b*. Local population differences in phenotypic plasticity: predator-induced changes in wood frog tadpoles. Ecological Monographs 72:77–93.

Relyea, R. A. 2003*a*. How prey respond to combined predators: a review and an empirical test. Ecology 84:1827–1839.

Relyea, R. A. 2003*b*. Predator cues and pesticides: a double dose of danger for amphibians. Ecological Applications 13:1515–1521.

Relyea, R. A. 2004*a*. Fine-tuned phenotypes: tadpole plasticity under 16 combinations of predators and competitors. Ecology 85:172–179.

Relyea, R. A. 2004*b*. Growth and survival of five amphibian species exposed to combinations of pesticides. Environmental Toxicology and Chemistry 23:1737–1742.

Relyea, R. A. 2004*c*. Synergistic impacts of malathion and predatory stress on six species of North American tadpoles. Environmental Toxicology and Chemistry 23:1080–1084.

Relyea, R. A. 2005*a*. The heritability of inducible defenses in tadpoles. Journal of Evolutionary Biology 18:856–866.

Relyea, R. A. 2005*b*. The impact of insecticides and herbicides on the biodiversity and productivity of aquatic communities. Ecological Applications 15:618–627.

Relyea, R. A. 2005*c*. The lethal impact of Roundup on aquatic and terrestrial amphibians. Ecological Applications 15:1118–1124.

Relyea, R. A. 2005*d*. The lethal impacts of Roundup and predatory stress on six species of North American tadpoles. Archives of Environmental Contamination and Toxicology 48:351–357.

Relyea, R. A. 2006. The impact of insecticides and herbicides on the biodiversity and productivity of aquatic communities: response. Ecological Applications 15:1527–1532.

Relyea, R. A. 2011. Amphibians are not ready for Roundup®. Pages 267–300 *in* J. Elliott, C. Bishop, and C. Morrisey, editors. Wildlife ecotoxicology—forensic approaches. Springer, New York, New York, USA.

Relyea, R. A., and J. R. Auld. 2004. Having the guts to compete: How intestinal plasticity explains costs of inducible defenses. Ecology Letters 7:869–875.

Relyea, R. A., and J. R. Auld. 2005. Predator- and competitor-induced plasticity: How changes in foraging morphology affect phenotypic trade-offs. Ecology 86:1723–1729.

Relyea, R. A., and J. T. Hoverman. 2006. Assessing the ecology in ecotoxicology: a review and synthesis in freshwater systems. Ecology Letters 9:1157–1171.

Relyea, R. A., and D. K. Jones. 2009. The toxicity of Roundup Original MAX® to 13 species of larval amphibians. Environmental Toxicology and Chemistry 28:2004–2008.

Relyea, R. A., and N. Mills. 2001. Predator-induced stress makes pesticide carbaryl more deadly to gray treefrog tadpoles (*Hyla versicolor*). Proceedings of the National Academy of Sciences USA 98:2491–2496.

Relyea, R. A., N. M. Schoeppner, and J. T. Hoverman. 2005. Pesticides and amphibians: the importance of community context. Ecological Applications 15:1125–1134.

Relyea, R. A., and E. E. Werner. 1999. Quantifying the relation between predator-induced behavior and growth performance in larval anurans. Ecology 80:2117–2124.

Sakamoto, M., K.-H. Change, and T. Hanazato. 2006. Inhibition of development of anti-predator morphology in the small cladoceran *Bosmina* by an insecticide: Impact of an anthropogenic chemical on prey–predator interactions. Freshwater Biology 51:1974–1983.

Schoeppner, N. M., and R. A. Relyea. 2005. Damage, digestion, and defense: The roles of alarm cues and kairomones for inducing prey defenses. Ecology Letters 8:505–512.

Schoeppner, N. M., and R. A. Relyea. 2009. Interpreting the smells of predation: How alarm cues and kairomones induce different prey defenses. Functional Ecology 23:1114–1121.

Semlitsch, R. D., D. C. Scott, and J. H. K. Pechmann. 1988. Time and size at metamorphosis related to adult fitness in *Ambystoma talpoideum*. Ecology 69:184–192.

Smith, D. C. 1987. Adult recruitment in chorus frogs: effects of size and date at metamorphosis. Ecology 68:344–350.

Solomon, K. R., and D. G. Thompson. 2003. Ecological risk assessment for aquatic organisms from over-water uses of glyphosate. Journal of Toxicology and Environmental Health—Part B—Critical Reviews 6:289–324.

Sudo, M., T. Kawachi, Y. Hida, and T. Kunimatsu. 2004. Spatial distribution and seasonal changes of pesticides in Lake Biwa, Japan. Limnology 5:77–86.

Teplitsky, C., H. Piha, A. Laurila, and J. Merilä. 2005. Common pesticide increases costs of antipredator defenses in *Rana temporaria* tadpoles. Environmental Science and Technology 39:6079–6085.

Thompson, D. G., B. F. Wojtaszek, B. Staznik, D. T. Chartrand, and G. R. Stephenson. 2004. Chemical and biomonitoring to assess potential acute effects of Vision® herbicide on native amphibian larvae in forest wetlands. Environmental Toxicology and Chemistry 23:843–849.

Tsui, M. T. K., and L. M. Chi. 2003. Aquatic toxicity of glyphosate-based formulations: Comparison between different organisms and the effects of environmental factors. Chemosphere 52:1189–1197.

Van Buskirk, J. 2002. A comparative test of the adaptive plasticity hypothesis: Relationships between habitat and phenotype in anuran larvae. American Naturalist 160:87–102.

Weis, J. S., G. Smith, T. Zhou, C. Santiago-Bass, and P. Weis. 2001. Effects of contaminants on behavior: Biochemical mechanisms and ecological consequences. BioScience 51:209–217.

Wojtaszek, B. F., B. Staznik, D. T. Chartrand, G. R. Stephenson, and D. G. Thompson. 2004. Effects of Vision® herbicide on mortality, avoidance response, and growth of amphibian larvae in two forest wetlands. Environmental Contamination and Toxicology 23:832–842.

SCIENTIFIC REPORTS

OPEN

SUBJECT AREAS:
AGRI-ECOLOGY
ENVIRONMENTAL SCIENCES

Received
10 March 2014

Accepted
20 June 2014

Published
9 July 2014

Correspondence and requests for materials should be addressed to J.G.Z. (johann.zaller@boku.ac.at)

Glyphosate herbicide affects belowground interactions between earthworms and symbiotic mycorrhizal fungi in a model ecosystem

Johann G. Zaller[1], Florian Heigl[1], Liliane Ruess[2] & Andrea Grabmaier[1]

[1]Institute of Zoology, University of Natural Resources and Life Sciences Vienna, Gregor Mendel Straße 33, 1180 Vienna, Austria, [2]Institute of Biology, Ecology Group, Humboldt-Universität zu Berlin, Philippstrasse 13, 10115 Berlin, Germany.

Herbicides containing glyphosate are widely used in agriculture and private gardens, however, surprisingly little is known on potential side effects on non-target soil organisms. In a greenhouse experiment with white clover we investigated, to what extent a globally-used glyphosate herbicide affects interactions between essential soil organisms such as earthworms and arbuscular mycorrhizal fungi (AMF). We found that herbicides significantly decreased root mycorrhization, soil AMF spore biomass, vesicles and propagules. Herbicide application and earthworms increased soil hyphal biomass and tended to reduce soil water infiltration after a simulated heavy rainfall. Herbicide application in interaction with AMF led to slightly heavier but less active earthworms. Leaching of glyphosate after a simulated rainfall was substantial and altered by earthworms and AMF. These sizeable changes provide impetus for more general attention to side-effects of glyphosate-based herbicides on key soil organisms and their associated ecosystem services.

Earthworms and arbuscular mycorrhizal fungi (AMF) are important components in temperate ecosystems, influencing nutrient cycling and overall ecosystem functioning[1,2]. Earthworms are considered to be ecosystem engineers because they shred and redistribute organic material in soil, increase soil penetrability for roots, thus improving overall soil fertility[3,4]. Because of their importance, earthworms have also been used as bioindicators of soil health and quality[1,5,6]. Mycorrhizal fungi form a symbiosis with over 80% of vascular plant species and are also considered keystone species in temperate ecosystems because of their influence on plant nutrient supply[7] and soil aggregation[8]. In arable soils AMF are the dominant root symbionts that sustain plant growth[9]. Mycorrhized plants commonly show a higher uptake of phosphorus and nitrogen, as the fungal mycelium has more efficient mechanisms for absorbing mineral nutrients than roots and by extending the root system enabling further exploration of the soil resources[5,10,11]. In return, host plants provide photoassimilates (predominantly glucose and fructose) that are converted to lipids by the fungus and used for carbon transport and storage[9,12]. Recently, the analysis of fatty acids as biochemical markers considerably improved our knowledge in AMF distribution and foraging activity in soil[13]. Thereby, the soil phospholipid fatty acid (PLFA) 16:1ω5 represents viable hyphal biomass, while the neutral lipid fatty acid (NLFA) reflects fungal storage reserves such as spores, vesicles and propagules[14,15]. Moreover, the ratio of 16:1ω5 NLFA to PLFA indicates fungal phenology such as senescence or active colonization phases[12,16].

Despite their important roles in ecosystems, our understanding on ecological interactions between earthworms and AMF is rather limited. The few studies investigating earthworm-AMF interactions suggest that the response patterns are dependent on the species involved; as a result effects range from additive, synergistic, antagonistic or no interactive effects[17–20]. Here we examined, whether the interactions between earthworms and AMF are affected by herbicide application. We experimented with two essential players in temperate soil ecosystems: the anecic, vertically burrowing earthworm *Lumbricus terrestris* (Linnaeus 1758) and the arbuscular mycorrhizal fungi *Glomus mosseae* (T.H. Nicolson & Gerd.). As a herbicide we used Roundup (RU), the most widely used pesticide worldwide[21] containing the active ingredient glyphosate. Glyphosate is a broad-spectrum, post-emergence, non-selective chemical that kills plants by affecting the shikimate-pathway during photosynthesis[22]. Generally, glyphosate is regarded as environmentally friendly due to its fast biodegradation and strong adsorption to soil particles[23]. However, there is mounting evidence that many amphibian species[24–26] and other wildlife[27] can be detrimentally affected by glyphosate-based herbicides.

Contrary to the wide use of glyphosate surprisingly little is known on potential side effects on interactions between key soil organisms such as earthworms or AMF. Glyphosate effects on earthworms vary from detrimental[28–30] to no effects[31–33], however, to what extent their interaction with other soil organisms is affected by glyphosate has never been investigated. Studies testing glyphosate effects on AMF show an inhibition of AM fungal spore germination and germ tube growth[34] or reduced mycorrhiza in soil[35], however only at concentrations greater than those recommended for field use. Several other reports show no effect of glyphosate on mycorrhiza when applied at recommended doses[36–40]. The fate of glyphosate in ecosystems is another aspect which has rarely been investigated[41,42]. While glyphosate sorbs strongly to soil minerals[43], leaching and soil erosion by water or wind can transport glyphosate from land to water environments[44]. This glyphosate leaching is assumed to be affected by earthworms and/or AMF. Earthworms maintain soil structure and foster macropores, which may influence water infiltration[45] and thereby increase glyphosate leaching. On the other hand, mycorrhiza could lead to stronger absorption of glyphosate by binding and enmeshing soil particles into larger aggregates[46].

To investigate interrelationships between herbicide application, earthworms and AMF we set up a full-factorial mesocosm greenhouse experiment. We planted the mesocosms with the leguminous forb white clover (*Trifolium repens* L.), which is frequently used as green manure in agriculture. Three hypotheses were tested. First, herbicide application will increase earthworm activity as an increased amount of dead plant material will be available as food for earthworms. Second, herbicide application will not affect AMF in soil because of the very plant-specific mode of symbiotic interaction. Third, herbicide-stimulated earthworm activity increases the preferential flow of rainwater through burrows and therefore increase leaching of glyphosate; whereas AMF counteract glyphosate leaching as they enhance soil aggregation. Such terrestrial model ecosystems have been proposed as an ideal tool to evaluate the effects of chemicals in soil ecosystems in order to achieve a greater realism in the ecotoxicological evaluation of chemicals to non-target organisms[47].

Results

Plants. *Trifolium* leaves were killed by the herbicide within several hours, whereas stolons remained partly green. Shoot biomass of *T. repens* at harvest was significantly reduced by earthworms ($F_{1;16}$ = 5.485, P = 0.032) but not significantly affected by RU application ($F_{1;16}$ = 2.529, P = 0.131) or AMF ($F_{1;16}$ = 0.220, P = 0.645; shoot biomass across AMF and RU treatments: −EW 23.724 ± 2.283 g, +EW 18.812 ± 3.169 g). Root biomass of *T. repens* was unaffected by RU ($F_{1;16}$ = 0.190, P = 0.668), AMF ($F_{1;16}$ = 0.682, P = 0.421) or earthworms ($F_{1;16}$ = 0.082, P = 0.778; root biomass across treatments: 1.775 ± 0.361 g).

Earthworms. Earthworm activity was similar across treatments prior to herbicide application with mean surface cast production of 1.5 ± 0.1 casts day^{-1} mesocosm^{-1} and 3.6 ± 0.4 moved toothpicks day^{-1} mesocosm^{-1}. Earthworm activity measured by toothpicks was marginally significantly lower after herbicide application ($F_{1;10}$ = 4.490, P = 0.060), however was not influenced by AMF ($F_{1;10}$ = 0.001, P = 0.977; Figure 1a+1b). Herbicide application reduced earthworm activity (toothpicks) in +AMF mesocosms ($F_{1;4}$ = 9.042, P = 0.040; Figure 1b) but had no influence on earthworm activity in −AMF mesocosms (Figure 1a). Earthworm activity measured by surface cast production was neither influenced by RU nor AMF (Figure 1).

Earthworm fresh mass at harvest was on average 72% of the initially added fresh mass; neither AMF inoculation ($F_{1;10}$ = 0.138, P =

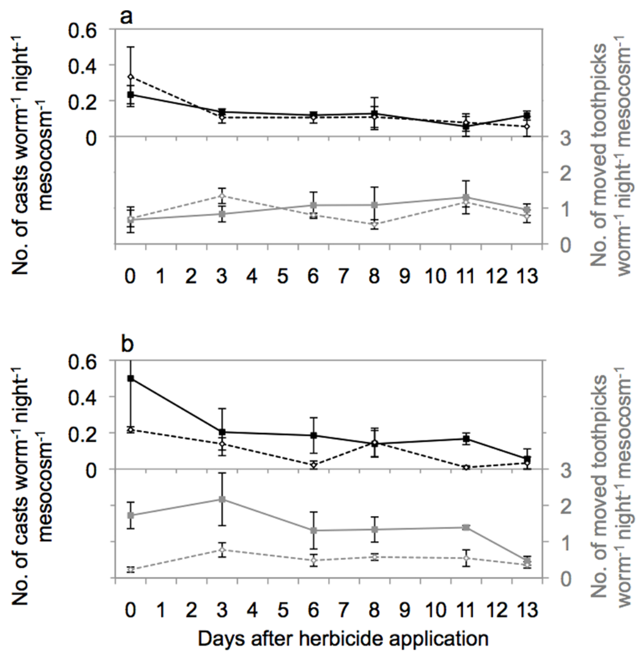


Figure 1 | Earthworm activity measured by surface cast production and moved toothpicks in mesocosms without (a) and with (b) AMF inoculation and without (continuous line) and with herbicide application (dotted line). Means ± SE, n = 3.

0.720) nor RU application ($F_{1;10}$ = 2.903, P = 0.127) affected recaptured earthworm fresh mass, but a significant AMF × RU interaction occurred ($F_{1;10}$ = 6.388, P = 0.035). Earthworm mass in the different treatments was: −RU/−AMF 11.0 ± 7.0 g, +RU/−AMF 9.5 ± 5.7 g; −RU/+AMF 5.3 ± 9.1 g, +RU/+AMF 16.4 ± 3.4 g. Earthworm activity (both moved toothpicks and surface castings) was not correlated to earthworm biomass or greenhouse mean air temperature or relative humidity (data not shown).

Mycorrhizae. Thirty-six weeks after AMF inoculation, average mycorrhization rates of *Trifolium* roots were 26% in +AMF and 15% in −AMF treatments. Across soil layers, herbicide application significantly reduced mycorrhization in +AMF ($F_{1;10}$ = 7.887, P = 0.023) but had no effect on mycorrhization rates in −AMF mesocosms (Figure 2). The reduction in mycorrhization due to herbicide application was even more pronounced when soil layers were considered separately (Table 1, Figure 2). In −RU/−EW mesocosms mycorrhization was significantly different between layer 0–5 cm and layer 5–10 cm ($F_{1;10}$ = 14.756, P = 0.018). In −RU + EW mesocosms mycorrhization differed significantly between layer 0–5 cm and layer 10–30 cm ($F_{1;10}$ = 23.093, P = 0.009). In +RU/−EW mesocosms mycorrhization differed significantly between layer 0–5 cm and layer 10–30 cm ($F_{1;10}$ = 20.050, P = 0.011). Earthworms had no influence on root AMF colonisation

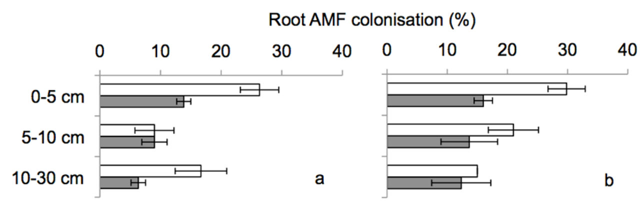


Figure 2 | Mycorrhization of *T. repens* roots in different soil layers without (a) and with (b) earthworms in mesocosms without (white) or with (grey) herbicide application. Means ± SE, n = 3.

Table 1 | ANOVA results on the effects of herbicide application (RU) and earthworms (EW) on *Trifolium repens* root AMF colonisation. ANOVA with * for P < 0.05, ** for P < 0.01, *** for P < 0.001

Soil layer	Roundup (RU)		Earthworms (EW)		RU × EW	
	F-value	P-value	F-value	P-value	F-value	P-value
0–5 cm	29.826	0.001**	1.381	0.274	0.076	0.789
5–10 cm	0.994	0.348	5.133	0.053	0.994	0.348
10–30 cm	3.870	0.085	0.430	0.530	1.346	0.279

across soil depths ($F_{1;10}$ = 2.575, P = 0.147; also no RU × EW interaction).

Hyphal biomass in soil assigned by 16:1ω5 PLFAs was not enhanced by AMF inoculation (Table 2, Figure 3). Highest PLFA concentrations of 16:1ω5 in soil were found in the layer 0–5 cm in mesocosms without any manipulation (−EW, −AMF, −RU). A significant herbicide AMF interaction occurred (no interaction between the three treatment factors). We found higher PLFA concentrations of 16:1ω5 in mesocosms with herbicide application, especially in combination with earthworms. AMF spores, vesicles and propagules assigned by 16:1ω5 in soil NLFAs were significantly enhanced by AMF inoculation (Table 2, Figure 3). Most storage reserves were found in mesocosms without any manipulation in layer 0–5 cm. Earthworms reduced the concentration of 16:1ω5 NLFAs and had a strong negative effect on storage structures of AMF assigned by NLFA/PLFA ratio (Table 2, Figure 3). This effect diminished in presence of herbicide, but was still visible. A herbicide-earthworm interaction occurred in layer 5–10 cm: means in NLFA concentration in −RU/−EW was higher than in +RU/+EW mesocosms (Figure 3).

Water infiltration and herbicide leaching. Water infiltration rate was unaffected by earthworms or AMF (Figure 4). Herbicide application showed a trend towards reduced water infiltration ($F_{1;22}$ = 3.796, P = 0.069; there was no Roundup × AMF interaction).

Concentration of glyphosate or its metabolite AMPA in the leachate was unaffected by earthworms or AMF (Figure 5). However, concentrations of glyphosate were significantly ($F_{1;10}$ = 7.572, P = 0.025) and of AMPA marginally significantly ($F_{1;10}$ = 4.515, P = 0.066) interactively affected by earthworms and AMF with increasing earthworm effects in −AMF and decreasing earthworm effects in +AMF mesocosms (Figure 5). In −AMF mesocosms earthworms significantly increased glyphosate leaching ($F_{1;4}$ = 9.439, P = 0.037).

Table 2 | ANOVA results for effects of Arbuscular mycorrhizal fungi (AMF), Earthworms (EW), Roundup (RU) and their interactions on PLFA amount of 16:1ω5 and NLFA amount of 16:1ω5 in different soil layers. ANOVA with * for P < 0.05, ** for P < 0.01, *** for P < 0.001

Parameter	PLFA		NLFA		NLFA : PLFA ratio	
	F	P	F	P	F	P
Soil depth 0–5 cm						
AMF	4.926	0.041*	0.296	0.594	0.046	0.832
EW	0.029	0.866	4.595	0.048*	5.445	0.033*
RU	1.154	0.299	0.064	0.803	0.520	0.481
AMF × EW	0.751	0.399	0.425	0.524	1.207	0.288
AMF × RU	9.069	0.008**	6.353	0.023*	2.730	0.118
EW × RU	5.957	0.027*	2.543	0.130	0.734	0.404
AMF × EW × RU	0.031	0.862	3.486	0.080	4.267	0.055
Soil depth 5–10 cm						
AMF	0.011	0.918	4.485	0.050*	4.165	0.058
EW	8.047	0.011*	4.399	0.052	13.135	0.002**
RU	0.470	0.503	2.241	0.154	3.448	0.082
AMF × EW	4.146	0.059	3.126	0.096	0.411	0.530
AMF × RU	4.522	0.049*	3.424	0.083	0.454	0.510
EW × RU	2.050	0.171	5.449	0.033*	2.346	0.145
AMF × EW × RU	0.948	0.345	0.038	0.849	0.522	0.480

Discussion

To our knowledge, this is among the first studies investigating the impact of a glyphosate-based herbicide on ecological interactions between a vertically burrowing earthworm species (*Lumbricus terrestris*) and symbiotic mycorrhizal fungi. Contrary to our hypothesis, Roundup did not stimulate but rather decrease earthworm activity, especially in mesocosms with AMF amendment. Also in the +AMF mesocosms, earthworm biomass was 50% higher after Roundup application, than in −AMF mesocosms. This suggests that over the short duration of our experiment, Roundup led to heavier earthworms that were less active at the surface, probably because there was abundant food in form of dead roots or AMF in the soil that precluded earthworms from foraging food from the surface. Other studies showed that earthworm biomass was unaffected by glyphosate-based herbicides for endogeic species[48], whereas in temperate epigeic species[30,49] and tropical earthworms strong mass loss after glyphosate application was found[50]. Studies investigating effects of Roundup on soil dwelling endogeic earthworm species (*Aporrectodea caliginosa*) found no alteration of the energy status after acute exposure[31]. Glyphosate had no effect on growth of *A. caliginosa* in a pot experiment where the herbicide was mixed with soil[51], in contrast, another study showed that glyphosate reduces the growth of *A. caliginosa* even at a rate lower than recommended by the manufacturer[52]. Surface dwelling, epigeic earthworms showed no avoidance of Roundup treated leaves (*Eisenia andrei*[32]) or response in their depth distribution (*E. fetida*[53]) but avoided glyphosate treated soil[28,29]. Previous studies found no influence of glyphosate on the survival rate in temperate earthworm species *Aporrectodea trapezoides*, *A. rosea*, *A. caliginosa* or *A. longa* populations[30,48], whereas a 50% reduction in mortality was found for the tropical earthworm species *Pheretima elongata*[54]. Effects of glyphosate had no influence on reproduction of *E. fetida*[49], whereas others reported a significant reduction of hatched cocoons in glyphosate treated soil for this species[29,30].

Two things are important to note, when evaluating our current results and previous results from the literature. First, we monitored the surface activity of earthworms over a period of only two weeks after Roundup application and therefore no conclusions on long-term effects, consequences for reproduction or changes in belowground activity can be derived from this study. Second, findings on herbicide effects on epigeic species such as *E. fetida* are important contributions when testing possible mode of actions in ecotoxicological tests, however they are of limited value when aiming to evaluate pesticide effects under field situations as these species preferably live in habitats with an abundant surface litter layer which is not the case in arable agroecosystems where these herbicides are applied.

We found a 40% reduction of mycorrhization after Roundup application in soils amended with the mycorrhizal fungi *G. mosseae*. This is in contrast to what we hypothesized, based on the allegedly fast biodegradation of the herbicide and the very plant-specific mode of action. We explain this mainly by direct and indirect influences. Roundup could have directly affected active metabolite production in the plant with detrimental effects on root AMF colonisation[38]. Indirect effects of Roundup on AMF could have affected the intraradical phase of AMF that has been shown to be sensitive to several host plant metabolites which regulate AMF abundance[55–57]. Mycorrhizal infection of maize, soybean and cotton was influenced by glyphosate in pasteurized soil but not in non-pasteurized soil[38]. Our soil mixture was steam-sterilized, but afterwards amended with a microbial wash from field soil, therefore only differing from field

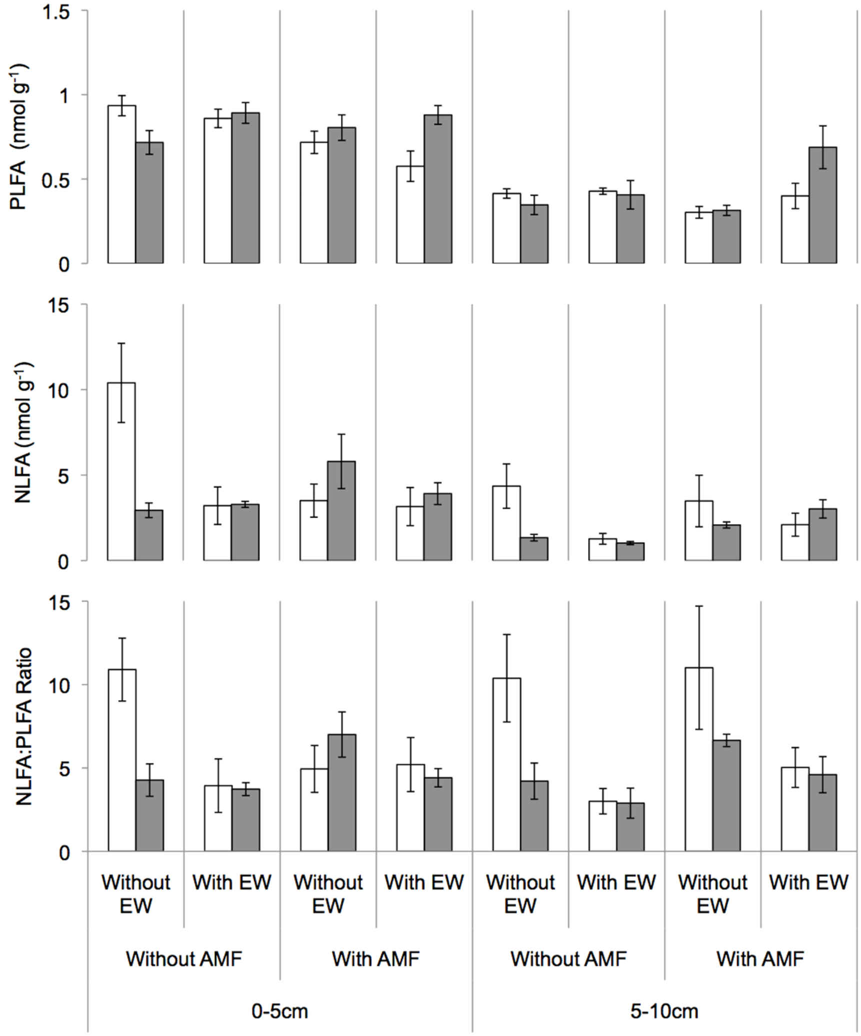


Figure 3 | PLFA amount of 16:1ω5, NLFA amount of 16:1ω5 in nmol g^{-1} DW soil and the ratio of 16:1ω5 NLFA to PLFA in different soil layers without (white) and with (grey) Roundup application, without/with earthworms in mesocosms without/with AMF inoculation. Mean ± SE, n = 3.

soil by the presence or absence of the inoculated AMF taxa. However, the latter did not enhance AMF hyphal biomass measured by 16:1ω5 PLFA, whereas spores assigned by fungal storage lipids, i.e. 16:1ω5 NLFA, were highest in soils without any manipulations (−AMF, −RU, −EW). These fungal propagules obviously have survived the steam-sterilization procedure. The generally low impact of soil amendment by the mycorrhiza inoculum points to competition with the indigenous soil community hampering the establishment of introduced *G. mosseae*. However, this cannot be assigned by the used biomarker fatty acid, 16:1ω5, as it is a measure for viable fungal hyphae biomass and storage fat in spores across the genus *Glomus*[15,58,59].

Direct influence of Roundup on AM fungi are generally regarded to be minor as soil fungi are well protected from direct contact with the herbicide. Indeed reports show rather insignificant influence of glyphosate on hyphal growth and germination of spores as well as root AMF colonisation[34,37,38,40,57]. However, in the present experiment Roundup application affected hyphal (i.e. amount of 16:1ω5 PLFA) and spore (i.e. amount of 16:1ω5 NLFA) biomass in the soil. Spore biomass generally declined with herbicide application, which is in accordance with others who showed reduced spore viability even under the lowest glyphosate rate[60]. Interestingly, the presence of earthworms resulted in a comparable negative effect on fungal storage structures. Earthworms are reported to influence AMF positively

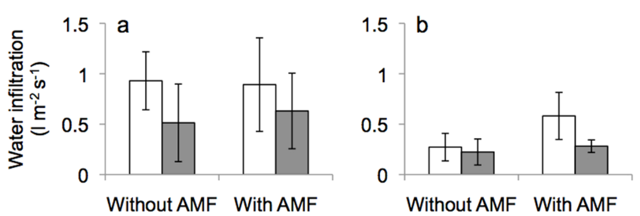


Figure 4 | Water infiltration rate measured in mesocosms without earthworms (a) and with earthworms (b) in response to AMF, without (white) and with (grey) Roundup application. Means ± SE, n = 3.

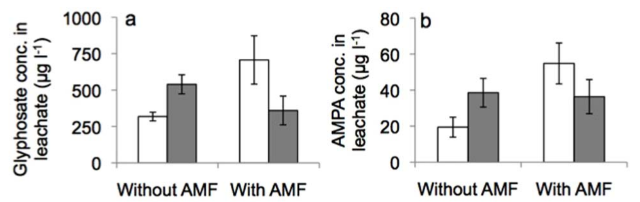


Figure 5 | Glyphosate concentration (a) and its metabolite AMPA (b) in soil leachate. Illustrated are concentrations in mesocosms with herbicide application, without and with AMF inoculation and without (white) and with (grey) earthworms. Means ± SE, n = 3.

by contribution to the dispersal of spores[61,62] yet our results indicate feeding rather than propagating fungal spores[63], which is supported by the corresponding changes in the NLFA to PLFA ratio. Spores, hyphae and infected root pieces form the three types of AMF propagules in soil, however their importance varies due to fungal species. In Glomeraceae, the extraradical mycelium is the most important source of inoculum, whereas spores are the main propagules in Gigasporaceae[64]. Thus, hyphae of *Glomus* are responsible for rapid colonization of new hosts, and hyphal biomass was positively affected by herbicide application especially in combination with earthworms. This indicates that glyphosate application alters fungal phenology, i.e. fosters fungal foraging over resting structures. Such effects likely are mediated through the modification of host plant physiology[57]. In sum the performance of AMF was distinctly altered by both Roundup and earthworms, albeit the impact varied with AMF propagule structures. Given the immense importance of AMF for plant nutrition and soil structure[9,46], these effects can have ramification for the functioning of ecosystems.

Will effects of herbicide on earthworms and mycorrhiza influence herbicide leaching? We found a tendency that water trickled away more slowly after a simulated heavy rainfall in mesocosms treated with Roundup as compared to those without Roundup application, however water infiltration rate was not influenced by earthworms or AMF. As Roundup had no effect on shoot and root mass of *T. repens*, we assume that after Roundup application dead plant material soaked up the excessive water and blocked the downflow of water. We hypothesized that earthworms increase glyphosate leaching by a preferential flow of contaminated rainwater through burrows, but also expected AMF to decrease glyphosate leaching by binding and enmeshing soil particles into larger aggregates[46,65]. Interestingly, earthworms significantly increased glyphosate leaching only in absence of AMF, while in presence of AMF earthworms tended to decrease glyphosate leaching. It remains to be tested whether this is due to glyphosate uptake and accumulation by AMF or whether these AMF hyphae might have been consumed by earthworms thus protecting glyphosate from leaching. Other studies have shown that glyphosate is mostly located in the earthworm mucus[31] which is smeared along the walls of earthworm burrows[66] and could therefore increase herbicide leaching. Furthermore, a rapid preferential transport for even strongly sorbing pesticides such as glyphosate and pendimethalin was demonstrated[44]. In contrast, AMF hyphae and other microorganisms could play a role in bonding glyphosate in the burrow walls. Biopore walls represent hot spots for microbial activity and pesticide mineralization[67]. The drilosphere of the burrows of *L. terrestris* therefore created an ideal habitat for a diverse microbial community. Many soil bacteria are known to degrade the organophosphonate glyphosate, e.g. dominant rhizosphere colonizer such as *Pseudomonas*[68], bulk soil inhabitants such as *Arthrobacter*[69], or symbiotic groups such as the family *Rhizobiaceae*[70]. A higher phosphorus transport via fungal hyphae was reported after glyphosate application for *G. mosseae*[71]. These processes in turn decreased glyphosate leaching as burrows transmitted clean water past the herbicide-containing soil matrix[72]. For the current results this could mean that without earthworms, water from the simulated heavy rainfall seeped through the whole soil matrix and thus absorbing glyphosate from the soil. The hyphae and other soil microorganisms absorbed glyphosate and so decreased glyphosate leaching; because the burrows transmitted clean water past the herbicide-containing soil matrix[72].

Taken together, our results show for the first time that Roundup can affect important interactions between earthworms and AMF, two of the most important soil organisms. While the short-term influence of Roundup on earthworms seem rather minor, the detrimental effects on AMF in roots and soil can have wide consequences for crop cultivation. Given AMFs and earthworms eminent role in plant nutrition, a glyphosate-induced decline in AM fungi would require more fertilization with economical and ecological consequences for farmland management. The finding that Roundup affects, together with earthworms and AMF, water infiltration requires more attention especially as climate change models prognosticate heavier rainfalls. Results of this study also highlight the importance of more complex experimental settings that investigate interactions of several species in order to better assess potential effects of pesticides on the environment.

Methods

Experimental setup. We conducted a full-factorial mesocosm experiment manipulating the three factors Earthworms (two levels: earthworm addition, +EW vs. no earthworms, −EW), AMF (two levels: AMF inoculation, +AMF vs. no AMF inoculation, −AMF) and Herbicide application (two levels: Roundup application, +RU vs. no Roundup application, −RU; more details on the individual treatments below). The experiment was conducted in December 2011 in a greenhouse of the University of Natural Resources and Life Sciences Vienna (BOKU), Austria. During the course of the experiment mean daytime air temperature inside the greenhouse was 20.1 ± 3.2°C at a mean relative humidity of 55.2 ± 5.4%; mean nighttime air temperature was 15.3 ± 2.6°C at a mean relative humidity of 68.3 ± 6.7%; to ensure optimal light conditions, three 1000-W Radium lamps (type HRI-T100W/D, WE-EF Leuchten, Bispingen, Germany) were installed in 1.5 m distance above the experimental units (14 hours light, 10 hours night).

We used 24 plastic pots (volume: 20 l, diameter: 31 cm, height: 30 cm; further called mesocosms) which were lined out with two layers of garden fleece at the bottom and extended at the upper rim with a 10 cm high barrier of transparent plastic to prevent earthworms from escaping; the fleece and barriers were also installed in mesocosms containing no earthworms to create similar microclimatic conditions among treatments.

Treatments. AMF treatments were prepared in March 2011 by first filling the mesocosms with 12 l steam-sterilized (3 hours at 100°C) field soil (Haplic Chernozem, silt loam) mixed with quartz sand (grain size 1.4–2.2 mm) in a ratio of 40 : 60 vol/vol. Characteristics of this soil mixture: $C_{org} = 24.1$ g kg^{-1}, $N_{tot} = 0.98$ g kg^{-1}, K = 111.2 mg kg^{-1}, P = 58.42 mg kg^{-1}, pH = 7.63. The upper 6 l of the +AMF treatments were filled with the same substrate mixture and amended with 25 g l^{-1} inoculum of *Glomus mosseae* (T.H. Nicolson & Gerd.; synonymously *Funneliformis mosseae* (T.H. Nicolson & Gerd.) C. Walker & A. Schüßler) obtained from a commercial supplier (Symbio-m Ltd., Lanskroun, Czech Republic). The −AMF controls were filled with the same amount of steam-sterilized and thus inactive AMF inoculum. We successfully used this substrate mixture in other experiments involving the same earthworm and AMF taxa[45,73,74]. Then 400 ml of microbial wash was added to each mesocosm to inoculate the steam-sterilized soil with microorganisms present in field soil[75]. This microbial wash contained 300 ml soil suspension (3500 g fresh soil dispensed in 7200 ml distilled H_2O filtered through a sieve-cascade from 2000 μm to 25 μm mesh size) and 100 ml AMF suspension (466 g AMF-inoculum dispensed in 2400 ml distilled H_2O filtered through the same sieve-cascade).

In April 2011 mesocosms were planted with white clover (*Trifolium repens* L.). Therefore, *T. repens* was first propagated from seeds in steam-sterilized potting soil, then 18 seedlings (average height about 10 mm, seedlings consisted of two cotyledons and two real leaves) were transplanted into each mesocosm in a regular hexagonal pattern with an equidistance to each other of 5 cm (240 seedlings m^{-2}). This seed material is commonly used by farmers in mixtures for green manuring and was obtained from the BOKU Department of Crop Sciences. No fertilizers were applied during the course of the experiment.

In December 2011 we added 4 adult individuals of vertically burrowing *Lumbricus terrestris* L. to the +EW mesocosms (16.6 ± 2.1 g mesocosm^{-1}, equivalent to 220.6 g m^{-2}). Earthworm densities were roughly oriented on the average earthworm biomass in temperate grasslands ranging between 52–305 g m^{-2} where 50–75% of the biomass consists of anecic species[1]. Earthworms were purchased from a local fishing bait shop. To acquaint earthworms with experimental conditions, we cultivated them in plastic boxes (climate chamber at 15°C) filled with steam-sterilized field soil and ground oat flakes as food before they were introduced to the mesocosms. Before earthworms were randomly added to the +EW mesocosms, they were washed free of attached soil, dried off on filter paper and weighed. All earthworms buried themselves in the soil within a few minutes. The mesocosms were randomly placed on greenhouse tables and randomly repositioned every second week to avoid treatment interactions with potential microclimatic gradients inside the greenhouse. No additional food was provided for earthworms in the mesocosms as there was abundant dead organic material on the soil surface. An automatic irrigation system added on average 0.5 l tap water day^{-1} to each mesocosm.

Herbicide was applied five days after earthworm insertion on half of the mesocosms comprising all treatment combinations. We used Roundup Speed (Monsanto Inc., St. Louis, Missouri, USA), a systemic, broad-spectrum herbicide containing 7.2 g l^{-1} of the active ingredient glyphosate. This herbicide is recommended for use in home and garden areas and was obtained from a garden center in Vienna. Following the instructions for use, we applied the herbicide directly onto the plants from the original bottle with the attached fine mist spray nozzle. We applied the herbicide once on day 5 after earthworm inserting at 4 p.m. without direct sunlight at an air temperature of 25°C. As recommended in the instruction text we sprayed the herbicide so that the plant surface was homogeneously covered and shiny from the herbicide film. This application needed 14 squirts of Roundup Speed with the spray nozzle mesocosm^{-1} amounting to 177.48 ml m^{-2}.

These treatments were replicated three times in a full-factorial design: two earthworm treatments × two AMF treatments × two RU treatments × three replicates equals totally 24 mesocosms.

Measurements and analyses. Earthworm activity was indirectly assessed during nighttime by 30 toothpicks mesocosm^{-1} that were vertically inserted (0.5 cm deep) in a consistent pattern. In the following morning the number of toothpicks differing from the original vertical position was considered as a measure of earthworm activity because earthworms crawl over the soil surface when searching for food. Knocked over toothpicks were counted as 1 and inclined toothpicks were counted as 0.5. As another measure of earthworm activity we additionally measured the number of freshly produced casts on the soil surface[76]. Both activity measurements were done parallel three times before and six times after herbicide application.

Water infiltration and Roundup leaching was measured seven days after the Roundup application by pouring 3 l of distilled water on top of the mesocosms simulating a rain shower of about 40 l m^{-2} (see also[45]). The time from pouring the water onto the mesocosms until the last water pool disappeared from the soil surface was recorded and used to calculate the water infiltration rate in l m^{-2} s^{-1}. We collected 250 ml of the leachate from the saucers at bottom of the mesocosms immediately stored it in a freezer at −20°C before it was analysed for glyphosate and its main metabolite aminomethylphosphonic acid (AMPA) in the laboratories of the BOKU Department of Forest and Soil Sciences using a HPLC-MS/MS method[77,78].

Harvest of the mesocosms started 14 days after herbicide application by cutting the remaining or untreated plants at the soil surface to obtain aboveground plant biomass production. Afterwards, soil was removed from the mesocosms in three separate layers 0–5 cm, 5–10 cm and 10–30 cm. Earthworms present in these soil layers were carefully washed free of soil, placed on moist filter paper, counted, weighed and released to the BOKU garden. Roots present in these soil layers were washed free of attached soil particles under a jet of tap water over a 1-mm sieve and sorted out. Dry mass of shoots and roots was determined after 48 hours oven-drying at 55°C. A portion of roots per layer was collected, cleared with boiling KOH for four minutes and stained for one minute with black Sheaffer ink[79]. Percentage of root length colonized by AMF considering arbuscules and vesicles (i.e. mycorrhization rate) was determined using the grid-line method by counting at least 100 intersections per sample[80].

Lipid extraction from pot soil was carried out by extracting 3–4 g of soil (wet weight) with Bligh & Dyer solvent (chloroform: methanol: citrate buffer as 1 : 2 : 0.8, pH 4)[81]. The obtained lipids were fractionated into neutral lipid (NLFAs), glycolipid and phospholipid fatty acids (PLFAs) on a silica column (HF Bond Elut - SI, Varian Inc.) by elution with chloroform, acetone and methanol, respectively. NLFAs and PLFAs were subjected to an alkaline methanolysis in 0.2 M methanolic KOH, and the fatty acid methyl esters (FAMEs) were extracted with hexane-chloroform. Samples were dissolved in isooctane and stored at −20°C until analysis. FAMEs were analyzed using an Agilent 7890A gas chromatograph equipped with a flame ionization detector (GC-FID) using a HP Ultra 2 capillary column (25 m × 0.2 mm i.d., film thickness 0.33 μm). The oven temperature program started with 170°C and increased by 28°C min^{-1} to 288°C, followed by 60°C min^{-1} to 310°C. FAMEs were identified with the Sherlock Pattern Recognition Software (MIDI®) by comparing retention times to a standard mixture, and quantifying based on the internal standard methylnondecanoate (19 : 0). To verify correct identification (chain length and saturation) a range of samples was additionally analyzed with the Agilent 7890A coupled to a Mass Selective Detector (Agilent 7000 Triplequadrupole) equipped with a HP5MS capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm), operated in splitless mode with helium as carrier gas. Oven temperature program started with 40°C and increased by 46°C min^{-1} to 200°C, followed by 5°C min^{-1} to 238°C, 120°C min^{-1} to 300°C. A mass range of 40–400 m/z was monitored in Scan mode. The fatty acid 16:1ω5 was applied as general marker for AMF, predominantly Glomales, with the PLFA fraction representing hyphal membranes and the NLFA fraction storage lipids[58,59].

Statistical analyses. All variables were tested for homogeneity of variances and normality using the tests after Levene and Kolmogorow-Smirnow, respectively. Data on PLFAs and NLFAs were log-transformed to meet the assumptions for parametric tests. We conducted a three way analysis of variance (ANOVA) to test the effects of Earthworms, AMF and Roundup on PLFAs, NLFAs, water infiltration and Roundup leaching. Here analyses for treatment effects on PLFAs and NLFAs were conducted for each soil layer separately. Earthworm activity (moved toothpicks and surface castings) during the course of the experiment was analyzed conducting a repeated measures ANOVA with Roundup and AMF as factors by only including data from mesocosms containing earthworms. Root AMF colonisation was analyzed for each soil layer using a two-way ANOVA considering the factors Earthworms and Roundup; mesocosms without AMF inoculation were not included. We also performed Pearson correlations between earthworm biomass and earthworm activity (moved toothpicks and surface castings) and between earthworm activity and mean air temperature or mean relative humidity. All statistical tests were performed in PASW Statistics 18 (vers. 18.0.0, IBM Corp., Armonk, New York, USA). Values given throughout the text are means ± SE.

1. Edwards, C. A. *Earthworm Ecology.* (CRC/Lewis Press, 1998).
2. Gianinazzi, S. *et al.* Agroecology: the key role of arbuscular mycorrhizas in ecosystem services. *Mycorrhiza* **20**, 519–530 (2010).
3. Syers, J. K. & Springett, J. A. Earthworms and soil fertility. *Plant Soil* **76**, 93–104 (1984).
4. Lavelle, P. Faunal activities and soil processes: Adaptive strategies that determine ecosystem function. *Adv Ecol Res* **27**, 93–132 (1997).
5. Gobat, J. M., Aragno, M. & Matthey, W. *The Living Soil: Fundamentals of Soil Science and Soil Biology.* (Science Publishers, 2004).
6. Paoletti, M. G. The role of earthworms for assessment of sustainability and as bioindicators. *Agric Ecosyst Environm* **74**, 137–155 (1999).
7. Cameron, D. D. Arbuscular mycorrhizal fungi as (agro)ecosystem engineers. *Plant Soil* **333**, 1–5 (2010).
8. Siddiky, M. R. K., Kohler, J., Cosme, M. & Rillig, M. C. Soil biota effects on soil structure: Interactions between arbuscular mycorrhizal fungal mycelium and collembola. *Soil Biol. Biochem.* **50**, 33–39 (2012).
9. Smith, S. E. & Read, D. J. *Mycorrhizal Symbiosis.* 3rd edn, (Academic Press, 2008).
10. Neumann, E. & George, E. Colonisation with the arbuscular mycorrhizal fungus *Glomus mosseae* (Nicol. and Gerd.) enhanced phosphorus uptake from dry soil in *Sorghum bicolor* (L.). *Plant Soil* **261**, 245–255 (2004).
11. Govindarajulu, M. *et al.* Nitrogen transfer in the arbuscular mycorrhizal symbiosis. *Nature* **435**, 819–823 (2005).
12. Gavito, M. E. & Olsson, P. A. Allocation of plant carbon to foraging and storage in arbuscular mycorrhizal fungi. *FEMS Microbiol Ecol* **45**, 181–187 (2003).
13. Ngosong, C., Gabriel, E. & Ruess, L. Use of the signature fatty acid 16:1ω5 as a tool to determine the distribution of arbuscular mycorrhizal fungi in soil. *J Lipids* **Article ID 236807**, 8 pages (2012).
14. Olsson, P. A. Signature fatty acids provide tools for determination of the distribution and interactions of mycorrhizal fungi in soils. *FEMS Microbiol Ecol* **29**, 303–310 (1999).
15. Olsson, P. A. & Johansen, A. Lipid and fatty acid composition of hyphae and spores of arbuscular mycorrhizal fungi at different growth stages. *Mycol Res* **104**, 429–434 (2000).
16. Olsson, P. A., Jakobsen, I. & Wallander, H. Foraging and resource allocation strategies of mycorrhizal fungi in a patchy environment. *Ecol Studies* **157**, 93–116 (2002).
17. Wurst, S., Dugassa-Gobena, D., Langel, R., Bonkowski, M. & Scheu, S. Combined effects of earthworms and vesicular–arbuscular mycorrhizas on plant and aphid performance. *New Phytol.* **163**, 169–176 (2004).
18. Eisenhauer, N. *et al.* Impacts of earthworms and arbuscular mycorrhizal fungi (*Glomus intraradices*) on plant performance are not interrelated. *Soil Biol. Biochem.* **41**, 561–567 (2009).
19. Milleret, R., Le Bayon, R. C. & Gobat, J. M. Root, mycorrhiza and earthworm interactions: their effects on soil structuring processes, plant and soil nutrient concentration and plant biomass. *Plant Soil* **316**, 1–12 (2009).
20. Zaller, J. G., Saccani, F. & Frank, T. Effects of earthworms and mycorrhizal fungi on the growth of the medicinal herb *Calendula officinalis* (Asteraceae). *Plant Soil Environ.* **57**, 499–504 (2011).
21. Woodburn, A. T. Glyphosate: production, pricing and use worldwide. *Pest Manag Sci* **56**, 309–312 (2000).
22. Duke, S. O. & Powles, S. B. Glyphosate: a once-in-a-century herbicide. *Pest Manag Sci* **64**, 319–325 (2008).
23. Vereecken, H. Mobility and leaching of glyphosate: a review. *Pest Manag Sci* **61**, 1139–1151 (2005).
24. Berger, G., Graef, F. & Pfeffer, H. Glyphosate applications on arable fields considerably coincide with migrating amphibians. *Sci. Rep.* **3**, 2622, doi:2610.1038/srep02622 (2013).
25. Brühl, C. A., Schmidt, T., Pieper, S. & Alscher, A. Terrestrial pesticide exposure of amphibians: An underestimated cause of global decline? *Sci. Rep.* **3**, 1135, doi:1110.1038/srep01135 (2013).
26. Relyea, R. A. The lethal impact of roundup on aquatic and terrestrial amphibians. *Ecol. Appl.* **15**, 1118–1124 (2005).
27. Köhler, H. R. & Triebskorn, R. Wildlife Ecotoxicology of Pesticides: Can We Track Effects to the Population Level and Beyond? *Science* **341**, 759–765, doi:10.1126/science.1237591 (2013).
28. Verrell, P. & Van Buskirk, E. As the worm turns: *Eisenia fetida* avoids soil contaminated by a glyphosate-based herbicide. *Bull Environm Contamin Toxicol* **72**, 219–224 (2004).
29. Casabé, N. *et al.* Ecotoxicological assessment of the effects of glyphosate and chlorpyrifos in an Argentine soya field. *J Soils Sediment* **8**, 1–8 (2007).
30. Correia, F. V. & Moreira, J. C. Effects of glyphosate and 2,4-D on earthworms (*Eisenia foetida*) in laboratory tests. *Bull Environm Contamin Toxicol* **85**, 264–268 (2010).
31. Bon, D. *et al.* In vivo 31P and 1H HR-MAS NMR spectroscopy analysis of the unstarved *Aporrectodea caliginosa* (Lumbricidae). *Biol. Fertil. Soils* **43**, 191–198 (2006).
32. Pereira, J. L. *et al.* Toxicity evaluation of three pesticides on non-target aquatic and soil organisms: commercial formulation versus active ingredient. *Ecotoxicol* **18**, 455–463 (2009).
33. Santos, M. J. G., Morgado, R., Ferreira, N. G. C., Soares, A. M. V. M. & Loureiro, S. Evaluation of the joint effect of glyphosate and dimethoate using a small-scale terrestrial ecosystem. *Ecotoxicol Environm Safety* **74**, 1994–2001 (2011).

34. Malty, J. D. S., Siqueira, J. O. & Moreira, F. M. D. S. Effects of glyphosate on soybean symbiotic microorganisms, in culture media and in greenhouse. *Pesquisa Agropecuária Brasileira* **41**, 285–291 (2006).
35. Ronco, M. G., Ruscitti, M. F., Arango, M. C. & Beltrano, J. Glyphosate and mycorrhization induce changes in plant growth and in root morphology and architecture in pepper plants (*Capsicum annuum* L.). *J Hortic Sci Biotechnol* **83**, 497–505 (2008).
36. Mujica, M., Fracchia, S., Ocampo, J. A. & Godeas, A. Influence of the herbicides chlorsulfuron and glyphosate on mycorrhizal soybean intercropped with the weeds *Brassica campestris* or *Sorghum halepensis*. *Symbiosis* **27**, 73–81 (1999).
37. Powell, J. R. *et al.* Effect of glyphosate on the tripartite symbiosis formed by *Glomus intraradices*, *Bradyrhizobium japonicum*, and genetically modified soybean. *Appl. Soil Ecol.* **41**, 128–136 (2009).
38. Savin, M. C., Purcell, L. C., Daigh, A. & Manfredini, A. Response of mycorrhizal infection to glyphosate applications and P fertilization in glyphosate-tolerant soybean, maize, and cotton. *J Plant Nutrition* **32**, 1702–1717 (2009).
39. Baumgartner, K., Fujiyoshi, P., Smith, R. & Bettiga, L. Weed flora and dormant-season cover crops have no effects on arbuscular mycorrhizae of grapevine. *Weed Research* **50**, 456–466 (2010).
40. Pasaribu, A., Mohamad, R. B., Awang, Y., Othman, R. & Puteh, A. Growth and development of symbiotic arbuscular mycorrhizal fungi, *Glomus mosseae* (Nicol. and Gerd.), in alachlor and glyphosate treated soils. *African J Biotechnol* **10**, 11520–11526 (2011).
41. Al-Rajab, A. J., Amellal, S. & Schiavon, M. Sorption and leaching of ^{14}C-glyphosate in agricultural soils. *Agron Sustain Developm* **28**, 419–428 (2008).
42. Laitinen, P., Rämö, S. & Siimes, K. Glyphosate translocation from plants to soil – does this constitute a significant proportion of residues in soil? *Plant Soil* **300**, 51–60 (2007).
43. Borggaard, O. K. & Gimsing, A. L. Fate of glyphosate in soil and the possibility of leaching to ground and surface waters: a review. *Pest Manag Sci* **64**, 441–456 (2008).
44. Kjær, J. *et al.* Transport modes and pathways of the strongly sorbing pesticides glyphosate and pendimethalin through structured drained soils. *Chemosphere* **84**, 471–479 (2011).
45. Zaller, J. G. *et al.* Earthworm-mycorrhiza interactions can affect the diversity, structure and functioning of establishing model grassland communities. *PLOS ONE* **6**, e29293 (2011).
46. Rillig, M. C. & Mummey, D. L. Mycorrhizas and soil structure. *New Phytol.* **171**, 41–53 (2006).
47. Edwards, C. A. Assessing the effects of environmental pollutants on soil organisms, communities, processes and ecosystems. *Europ. J. Soil Biol.* **38**, 225–231 (2002).
48. Dalby, P. R., Baker, G. H. & Smith, S. E. Glyphosate, 2,4-DB and dimethoate: Effects on earthworm survival and growth. *Soil Biol. Biochem.* **27**, 1661–1662 (1995).
49. Yasmin, S. & D'Souza, D. Effect of pesticides on the reproductive output of *Eisenia fetida*. *Bull Environm Contamin Toxicol* **79**, 529–532 (2007).
50. García-Pérez, J. A., Alarcón-Gutiérrez, E., Perroni, Y. & Barois, I. Earthworm communities and soil properties in shaded coffee plantations with and without application of glyphosate. *Appl. Soil Ecol.*, doi: 10.1016/j.apsoil.2013.1009.1006 (2013).
51. Martin, N. A. The effect of herbicides used on asparagus on the growth rate of the earthworm *Allolobophora caliginosa*. *Proc New Zealand Weed Pest Control Conf* **35**, 328–331 (1982).
52. Springett, J. A. & Gray, R. A. J. Effect of repeated low doses of biocides on the earthworm *Aporrectodea caliginosa* in laboratory culture. *Soil Biol. Biochem.* **24**, 1739–1744 (1992).
53. Santos, M. J. G., Morgado, R., Ferreira, N. G. C., Soares, A. M. V. M. & Loureiro, S. Evaluation of the joint effect of glyphosate and dimethoate using a small-scale terrestrial ecosystem. *Ecotoxicol Environm Safety* **74**, 1994–2001 (2011).
54. Morowati, M. Histochemical and histopathological study of the intestine of the earthworm (*Pheretima elongata*) exposed to a field dose of the herbicide glyphosate. *Environmentalist* **20**, 105–111 (2000).
55. Medina, M. J. H. *et al.* Root colonization by arbuscular mycorrhizal fungi is affected by the salicylic acid content of the plant. *Plant Science* **164**, 993–998 (2003).
56. Campos-Soriano, L., García-Garrido, J. M. & Segundo, B. S. Activation of basal defense mechanisms of rice plants by *Glomus intraradices* does not affect the arbuscular mycorrhizal symbiosis. *New Phytol.* **188**, 597–614 (2010).
57. Sheng, M., Hamel, C. & Fernandez, M. Cropping practices modulate the impact of glyphosate on arbuscular mycorrhizal fungi and rhizosphere bacteria in agroecosystems of the semiarid prairie. *Can J Microbiol* (2012).
58. Olsson, P. A., Bääth, E., Jakobsen, I. & Söderström, B. The use of phospholipid and neutral lipid fatty acids to estimate biomass of arbuscular mycorrhizal fungi in soil. *Mycol Res* **99**, 623–629 (1995).
59. Olsson, P. A., Larsson, L., Bago, B., Wallander, H. & Arle van, I. M. Ergosterol and fatty acids for biomass estimation of mycorrhizal fungi. *New Phytol.* **159**, 1–20 (2003).
60. Druille, M., Omacinia, M., Golluscio, R. A. & Cabello, M. N. Arbuscular mycorrhizal fungi are directly and indirectly affected by glyphosate application. *Appl. Soil Ecol.* **72**, 143–149 (2013).
61. Gange, A. C. Translocation of mycorrhizal fungi by earthworms during early succession. *Soil Biol. Biochem.* **25**, 1021–1026 (1993).
62. Lawrence, B., Fisk, M. C., Fahey, T. J. & Suarez, E. R. Influence of nonnative earthworms on mycorrhizal colonization of sugar maple (*Acer saccharum*). *New Phytol.* **157**, 145–153 (2003).
63. Bonkowski, M., Griffiths, B. S. & Ritz, K. Food preferences of earthworms for soil fungi. *Pedobiologia* **44**, 666–676 (2000).
64. Schalamuk, S. & Cabello, M. Arbuscular mycorrhizal fungal propagules from tillage and no-tillage systems: possible effects on Glomeromycota diversity. *Mycologia* **102**, 261–268 (2010).
65. Daynes, C. N., Field, D. J., Saleeba, J. A., Cole, M. A. & McGee, P. A. Development and stabilisation of soil structure via interactions between organic matter, arbuscular mycorrhizal fungi and plant roots. *Soil Biol. Biochem.* **57**, 683–694 (2013).
66. Jégou, D., Schrader, S., Diestel, H. & Cluzeau, D. Morphological, physical and biochemical characteristics of burrow walls formed by earthworms. *Appl. Soil Ecol.* **17**, 165–174 (2001).
67. Badawi, N., Johnsen, A. R., Brandt, K. K., Sørensen, J. & Aamand, J. Hydraulically active biopores stimulate pesticide mineralization in agricultural subsoil. *Soil Biol. Biochem.* **57**, 533–541 (2013).
68. Jacob, G. S. *et al.* Metabolism of glyphosate in Pseudomonas sp. strain LBr. *Appl Environ Microbiol* **54**, 2953–2958 (1988).
69. Pipke, R., Amrhein, N., Jacob, G. S., Schaefer, J. & Kishore, G. M. Metabolism of glyphosate in an Arthrobacter sp. GLP-1. *Eur J Biochem* **165**, 267–273 (1987).
70. Liu, C.-M., McLean, P. A., Sookdeo, C. C. & Cannon, F. C. Degradation of the herbicide glyphosate by members of the family rhizobiaceae. *Appl Environ Microbiol* **57**, 1799–1804 (1991).
71. Pasaribu, A. *et al.* Effect of herbicide on sporulation and infectivity of vesicular mycorrhizal (*Glomus mosseae*) symbiosis with peanut plant. *J Animal Plant Sci* **32**, 1671–1678 (2013).
72. Farenhorst, A., Topp, E., Bowman, B. T. & Tomlin, A. D. Earthworm burrowing and feeding activity and the potential for atrazine transport by preferential flow. *Soil Biol. Biochem.* **32**, 479–488 (2000).
73. Zaller, J. G., Frank, T. & Drapela, T. Soil sand content can alter effects of different taxa of mycorrhizal fungi on plant biomass production of grassland species. *Europ. J. Soil Biol.* **47**, 175–181 (2011).
74. Zaller, J. G. *et al.* Subsurface earthworm casts can be important soil microsites specifically influencing the growth of grassland plants. *Biol. Fertil. Soils* **49**, 1097–1107 (2013).
75. Koide, R. T. & Li, M. Appropriate controls for vesicular-arbuscular mycorrhiza research. *New Phytol.* **111**, 35–44 (1989).
76. Zaller, J. G. & Arnone, J. A. Activity of surface-casting earthworms in a calcareous grassland under elevated atmospheric CO_2. *Oecologia* **111**, 249–254 (1997).
77. Popp, M. *et al.* Determination of glyphosate and AMPA in surface and waste water using high-performance ion chromatography coupled to inductively coupled plasma dynamic reaction cell mass spectrometry (HPIC-ICP-DRC-MS). *Analyt Bioanalyt Chem* **391**, 695–699 (2008).
78. Todorovic, G. *et al.* Determination of glyphosate and AMPA in three representative agricultural Austrian soils with a HPLC-MS/MS method. *Soil Sediments Contamin* **22**, 332–350 (2013).
79. Vierheilig, H., Coughlan, A. P., Wyss, U. & Piche, Y. Ink and vinegar, a simple staining technique for arbuscular-mycorrhizal fungi. *Appl Environ Microbiol* **64**, 5004–5007 (1998).
80. Giovanetti, M. & Mosse, B. An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. *New Phytol.* **84**, 489–500 (1980).
81. Frostegård, A., Tunlid, A. & Bååth, E. Phospholipid fatty acid composition, biomass and activity of microbial communities from two soil types experimentally exposed to different heavy metals. *Appl Environm Microbiol* **59**, 3605–3617 (1993).

Acknowledgments

We are grateful to Alina Schmerbauch, Claudia Lichtenegger, Bernadett Handl, Elsa Ferstl, Thomas Müllner and Norbert Schuller for help in the greenhouse and laboratory, to Peter Liebhard for providing the seed material. Alexander Bruckner supported and Axel Mentler conducted the glyphosate and AMPA analyses. Pia Euteneuer and Karl Refenner from the BOKU Department of Applied Plant Sciences and Plant Biotechnology are acknowledged for providing logistical support.

Author contributions

Conceived and designed the experiment: F.H., A.G., J.G.Z. Performed the experiment: F.H., A.G., J.G.Z. Analyzed the data: F.H., J.G.Z., L.R. Contributed reagents/materials/analysis tools: L.R. Wrote the paper: J.G.Z., F.H., L.R., A.G.

Additional information

Competing financial interests: The authors declare no competing financial interests.

How to cite this article: Zaller, J.G., Heigl, F., Ruess, L. & Grabmaier, A. Glyphosate herbicide affects belowground interactions between earthworms and symbiotic mycorrhizal fungi in a model ecosystem. *Sci. Rep.* **4**, 5634; DOI:10.1038/srep05634 (2014).

Final Report

Environmental decay of glyphosate in broom-infested Mt. Tamalpais soils and its transport through stormwater runoff and soil column infiltration

Submitted to

Marin Municipal Water District
Corte Madera, CA 94925

Submitted by

Hyun-Min Hwang and Thomas M. Young
Environmental Quality Laboratory
Department of Civil and Environmental Engineering
University of California, Davis

April 19, 2011

INTRODUCTION

Wildfires can cause serious damage to biological diversity and structure and can also promote significant erosion that reduces the capacity of reservoirs and degrades the quality of water that provides drinking water. To manage and reduce the risks of wildfires, Marin Municipal Water District (MMWD) is currently updating its Vegetation Management Plan (VMP) that was originally adopted in 1994. The VMP also addresses concerns about degradation of habitat and biological resources in the District's watersheds. One of the severe threats is expansion of invasive weeds (e.g., *Genista monspessulana*) that provide fuels for wildfire and disturb ecological health. A part of this update is to identify feasible and safe methods of controlling weeds in the Mt. Tamalpais watersheds. Currently, chemical weed control by application of herbicides is being considered as one of the effective and cost-efficient weed management actions. However, due to possible impacts of herbicides on the health of humans and wildlife, considerable concerns about chemical weed control have arisen.

Recently, MMWD is considering using a mixture of Aquamaster and Competitor to control weeds. Glyphosate is the active ingredient of Aquamaster that does not contain surfactants. Competitor is a mixture of surfactants (98% ethyl oleate) that are designed to increase the effects of herbicides. When herbicides are mixed with surfactants, typically they are more bioavailable and thus can be degraded more rapidly by microorganisms. Surfactants may also increase wash-off of soil bound glyphosate and its soil column infiltration rates. Reported environmental half-lives of glyphosate in forest soils range mostly between 10 and 60 days (Feng and Thompson, 1990; Newton et al., 1994; WHO, 1994), depending on field conditions such as microbial activity, foliage litter coverage, and soil moisture content. Some studies reported even longer half-lives of up to 2 years (WHO, 1994). This wide variability of literature half-life values hampers MMWD from developing protective herbicide application strategies regarding the timing and rates of herbicide application for weed control. During winter, when frequent precipitation is expected, especially in northern California, residues of glyphosate can be washed away from the application areas by stormwater runoff that may enter receiving water bodies providing drinking water. Groundwater can also be contaminated through soil column infiltration of glyphosate. MMWD is considering an herbicide application window (July 15 through September 15) to minimize possible wash off by stormwater runoff, if the herbicide application is adopted for weed control in the future in the Mt. Tamalpais watersheds. Herbicide mixtures applied at different portions of this window may decay at different rates because ambient environmental conditions are different. Herbicide mixtures applied in the late portion of the application window may not be degraded below safe levels before stormwater runoff washes them away from the application areas. However, no systematic research regarding the persistence of glyphosate and its potential impacts on the quality of surface water and groundwater has been conducted in the Mt. Tamalpais watersheds.

This study was designed to investigate the decay of glyphosate in broom-infested soil in Mt. Tamalpais. Decay rates of glyphosate will provide critical information required to decide appropriate application timing to minimize any adverse effects of glyphosate. This study also investigated transport of glyphosate through stormwater runoff and soil infiltration. Due to limited budget, this study tested worst-case scenarios. The results will be incorporated into the existing risk assessment model built previously for the District.

BACKGROUND

Glyphosate (N-phosphonomethylglycine) is a post-emergent and non-selective organophosphorus herbicide that is widely used to control weeds in agricultural, aquatic, forestry, and residential settings. Octanol-water partition coefficients (log K_{ow}) of glyphosate vary from –2.8 to –3.5, indicating that its bioaccumulation potential is very low. Detailed information about environmental fate and toxicity of glyphosate are available in reports published by WHO (1994), OEHHA (2007), and MMWD (2008).

Once glyphosate reaches soil, typically it is strongly adsorbed onto the soil forming insoluble complexes with soil cation exchange sites. Major environmental dissipation processes include microbial degradation, hydrolysis, and photolysis. Due to the low vapor pressure of glyphosate, loss through evaporation is minimal. Glyphosate is mainly degraded to AMPA that is eventually transformed to inorganic constituents, including phosphate and carbon dioxide. The environmental half-life of glyphosate in soils typically ranges from 10 to 174 days (WHO, 1994), depending on soil and climate conditions. AMPA is equally or less stable in the environment and less toxic than glyphosate.

Although the water solubility of glyphosate is high (12 g/L), glyphosate mainly exist in a particle bound form in aqueous solutions because of its relatively high solid-water partition coefficients (K_d), between 5,000 and 340,000 L/kg. This distribution coefficient indicates that, in the aqueous phase, glyphosate preferentially binds to soil particles and thus in flowing water such as stormwater runoff, particles are likely a major vector carrying glyphosate. Precipitation, soil composition, drainage type, and other parameters influence the leaching of glyphosate from soil. Field and laboratory studies indicate that glyphosate generally does not move vertically in the soil below the topmost 15 cm soil layer (U.S. EPA, 1993).

There are large amounts of data on potential acute health effects related to human exposure to glyphosate. Serious poisonings are rare because glyphosate is not well absorbed through the skin or by inhalation, the most common routes of exposure. The California Office of Environmental Health and Hazard Assessment (OEHHA, 2007) reported a public health goal of 0.9 mg/L (900 ppb) for glyphosate in drinking water. They concluded that this public health goal provides adequate protection for the general population and potential sensitive subpopulations such as pregnant women and their fetuses, infants, and the elderly.

SAMPLE COLLECTION AND CHEMICAL ANALYSIS

Study sites

Total 6 sites (Figure 1) were selected for the present study. Three sites (A, B, and C) were selected to investigate potential wash-off and transportation by stormwater runoff and soil infiltration of glyphosate. Glyphosate application area for both sites A and B was 30 feet by 30 feet and smaller for site C (10 feet by 20 feet). For the sites A and B, the buffer zone was 30 feet. Under the draft, revised VMP, MMWD is considering buffer zones (at least 100 feet from creeks, streams, and reservoirs used for drinking water production), in which herbicides will not be applied to minimize any possible input of herbicides to surface water through stormwater runoff. If no herbicide is detected in stormwater runoff collected for the present study with narrower buffer zone, we can assume that herbicide will not be detected in settings with wider buffer zones. Environmental settings of the sites A and B are very different. The site A is within a relatively flat area and covered by a thin layer of plant litter, while the site B is within a relatively steep area and covered by a thick layer of plant litter, which could hold substantial amounts of water. Site C is within a flat, densely shaded area immediately adjacent to a newly constructed retaining wall where native soils were disturbed and amended with unconsolidated fill material during construction. The environmental settings of sites A, B, and C are typical for broom-infested sites in the Mt. Tamalpais watershed.

Originally, the sites A and B were also supposed to be used for glyphosate degradation test. However, there was rainfall about a week after the application, which is very unusual at this time of year. Because the glyphosate degradation might be accelerated by the extra moisture supplied by this unexpected rain, we decided the environmental conditions did not represent typical conditions during the application window and thus we didn't continue glyphosate degradation study in summer of 2009.

In the summer of the following year, two additional sites (D and E) were selected to investigate the degradation of glyphosate applied to broom leaves. Site D was selected as mostly exposed to sunlight and site E was selected as mostly shaded site, but it turned out that the site D was exposed to sunlight in the morning and shaded in the afternoon and vise versa for the site E. To minimize sampling errors resulting from an uneven spray pattern (see below), one site (F) near the Sky Oaks Ranger Station was selected for degradation of glyphosate in surface soil. This site had been shaded always and thus degradation by direct UV radiation was likely to be negligible.

Glyphosate application

MMWD is considering using a mixture of Aquamaster and Competitor to control weeds and thus the same herbicide mixture (2% Aquamaster, 3% Competitor, and 95% water) was applied to each site at a maximum rate (2 quarts per acre). Glyphosate is the active ingredient of Aquamaster that does not contain surfactants. Competitor is a mixture of surfactants (98% ethyl oleate) that are designed to increase the effects of herbicides. A blue dye (Blazon) was also added to the mixture as an indicator to show application patterns and application areas. Application method was targeted spraying onto individual plants with a backpack sprayer. For this reason, initial glyphosate concentrations in the surface soil were expected to be very heterogeneous. To account for errors that might be caused by sampling in the heterogeneous environment, the mixture was sprayed again on a separate site (F) where the application could be controlled. For this soil half-life study, the herbicide mixture was sprayed evenly.

Sample collection

Surface stormwater runoff samples were collected at the bottom end of the buffer zone (30 feet) of the sites A and B for three rain events. Two events were natural rain events and one event was artificial rain event simulated by spraying tap water with the help of a fire truck. For the two natural rain events, two pre-cleaned stainless cans (40 L) were installed on the ground for each site a few days prior to rainfalls. The cans were retreated and transported to the laboratory after the rainfalls. No leaves were collected from the ground. Upon arrival at the laboratory, the stormwater runoff samples were filtered using glass fiber filters (Whatmann GF/F, 0.7 um pore size). Filtered water samples and particles on the filter papers were stored in a cold room (4 °C) and a freezer (-20 °C), respectively, until chemical analysis was performed. We were not able to collect any runoff samples from the site B from natural events, though this site is within a relatively steep area, because the surface of this area was covered by a thick layer of plant litters, which could hold substantial amount of water. Since this type of environmental setting is common in the Mt. Tamalpais watershed, transportation by direct runoff early in the rainy season when soils are not saturated is expected to be minimal. When the artificial rain event was applied to this site, even about 15,000 L of tap water sprayed to the site B for two hours (equivalent to 215 cm/day (= 7 ft/day) of rainfall), failed to produce any runoff.

Soil core samples (3 cores per each application plot) were collected using PVC pipes (3.18 cm ID ´ 60 cm long) in June 2010 to investigate the first year soil infiltration of glyphosate. Six additional soil cores were collected from the application site C to perform laboratory infiltration simulation study. All core samples were transported to the laboratory immediately. The cores for the first year infiltration were sliced by 5cm interval and stored in a freezer (-20 °C) until chemical analysis was performed. Because the core soils were compressed while pushing the pipes down through the soil, we marked the depth of core pipes pushed down through the soil and actual soil core depth inside the pipes to calculate soil core compression. Actual depth of the 5 cm of the collected soil layer was turned out to be 6 cm.

Broom leaves were collected from the application sites D and E over 87 days (June 10, 2010-August 30, 2010) with one or two weeks of interval. Broom leaves were randomly collected from at least 10 stems and placed in pre-cleaned aluminum foil and Ziploc bags. Blue dye could be observed clearly only up to two weeks until the broom leaves were still greenish yellow, so the blue dye could be used only as a short-term indicator. When the sites were visited again for sampling two weeks after the application, most broom died (Figure 2). Some broom remained alive up to 7 weeks after the application presumably because the amount of the herbicide mixture they received was insufficient to kill them. Visual inspection indicated that a significant fraction of dead leaves were still attached on the stems and branches and some dead leaves were detached and fell to the ground. No attempts were made to quantify fractions of detached leaves. For each sampling, stems or branches with dead leaves were collected. No leaves were collected from the ground. Collected samples were transported immediately to the laboratory and stored in a freezer (-20 °C) until chemical analysis was performed.

Surface soil samples were collected from the application site F over 80 days (June 17, 2010-August 30, 2010) with one or two weeks of interval. For each collection, three replicate of the top surface soils (0-0.5 cm) were collected in Teflon tubes. Each collection spot was marked after collection to avoid collecting soils from the spots previously collected. Collected samples were transported immediately to the laboratory and stored in a freezer (-20 °C) until chemical analysis was performed.

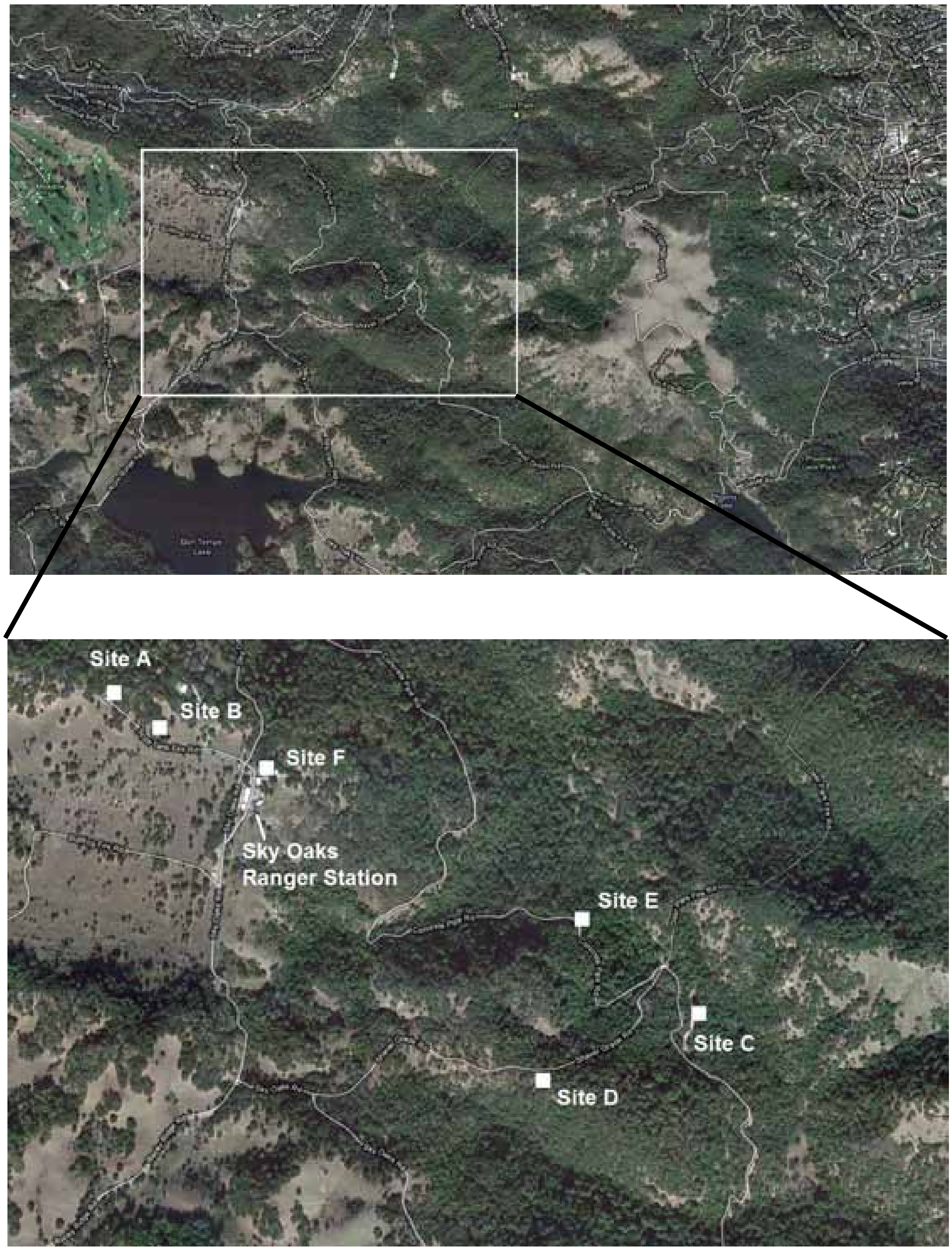


Figure 1. Study sites

Day 0

Week 1

Week 2

Week 3

Week 5

Week 7

Figure 2. Changes of broom leaves after the application of the herbicide mixture (glyphosate plus surfactant) in summer of the year 2010.

Laboratory infiltration simulation

For the laboratory infiltration simulation, 200 mL of de-ionized Milli-Q water was added to each of three cores collected in June 2010 from the site C. The volume of water added to the cores is equivalent to 25 cm of rainfall. The added water was allowed to move down through the soil cores by gravity for three days until no dripping from the bottom of the cores was observed. The cores were sliced by 5 cm interval and stored in a freezer (-20 °C) until chemical analysis was performed.

Sample analysis

Solid samples were analyzed using a modification of the method (Huang et al., 2004) reported by our laboratory. Stormwater samples were analyzed using a modification of the method reported by U.S. Geological Survey (Lee et al., 2002). To extract glyphosate in water samples, 1 mL of filtered water and 100 μL of surrogate solution containing ^{13}C-labled glyphosate and ^{13}C-labled AMPA was placed in 5 mL glass vials. Solution in the vials was concentrated to dryness using nitrogen gas. The target compounds were then redissolved with derivatization agents, 400 μL of TFF (2,2,2-trifluoroethanol) and 800 μL TFAA (trifluoroacetic anhydride), to change the target compounds into more volatile forms, which can be analyzed by gas chromatography. The vials were placed on a hot plate (80 ºC) for 1 hour to enhance derivatization. After the solutions were evaporated to dryness under nitrogen, they were redissolved with 1 mL of ethyl acetate. The extracts were transferred into 2 mL GC vials and internal standard (d_{10}-pyrene) was added.

To extract glyphosate in soil samples, about 5 g (fresh weight) of homogenized soil, and 100 μL of surrogate solution, and 10 mL of aqueous solutions containing 0.25 M NH_4OH and 0.1 M KH_2PO_4 were added into Teflon vials. After 1 hour of extraction in a sonication bath, the vials were shaken on a rotary tumbler for 24 hours. After centrifugation at 2500 rpm for 30 minutes, the supernatant was transferred into 20 mL vials. The samples were extracted again with 10 mL of the aqueous solution and the supernatant was combined together. One mL of extracts was transferred into 5 mL glass vials and processed using the method identical to that used for water samples as described above. To extract target compounds in broom leaves, about 150 leaves were placed in Teflon vials and processed using the same procedure used for the soil samples.

To measure water content, about 1 g of soil and leave samples were dried in an oven (60 ºC) for 24 hours. Derivatized glyphosate and AMPA were identified and quantified using a GC-MS (Agilent 6890 GC and Agilent 5973 MSD) equipped with an Agilent DB-5MS column (30 m x 0.25 mm, 0.25 μm film thickness). The injector temperature was 240 ºC. The initial oven temperature was 70 ºC and increased to 240 ºC at 15 ºC/min and held for 5 min. The mass selective detector was operated in EI (electron impact ionization) mode and SIM (selective ion monitoring) mode. All reported concentrations are dry weight basis. For QA/QC, each batch of the samples included a laboratory procedural blank and duplicate sample. Glyphosate and AMPA were not detected in all blank samples. Laboratory procedural blank samples contained only extraction solutions and surrogate compounds and were processed in the same way as that used for the field samples. Any detection of target compounds in blank samples indicates that samples are contaminated in the laboratory by unknown sources and target compounds detected in field samples might be also linked to laboratory contamination. Relative percent differences of the duplicate samples were less than 30%. To quantify target compound concentrations more accurately, isotope labeled surrogate standards were spiked to all samples. Recoveries of surrogate standards were variable, ranging from 40 to 95%, which is commonly observed when target compounds need to be derivatized for GC analysis. Concentrations of target compounds in all samples were adjusted using the surrogate recovery percent. When surrogate recovery is 80%, then target compound recovery is also assumed to be 80%. It indicates that 20% of target compounds were not derivatized and/or lost while the samples were processed in the laboratory. In this case, the final concentration is adjusted for the loss (20%), which is a standard procedure that should be followed to analyze environmental samples for organic compounds. If no surrogate standards are used, especially when the target compounds need to be derivatized, analytical results are significantly less reliable.

RESULTS AND DISCUSSION

Half-life in soil

Changes in glyphosate and AMPA concentrations in the surface soils are presented in Figure 3. Initial AMPA concentration was much lower than glyphosate concentrations and AMPA concentrations declined almost in the same rate as that of glyphosate. Other studies also found the same pattern (Feng and Thompson, 1990; Newton et al., 1994). Considering much lower concentrations and toxicity, environmental impacts of AMPA is likely to be negligible. Their half-lives in soil were calculated using a first-order degradation equation. Half-life of glyphosate in soil was 44 days, which is within the range (30 to 60 days) typically reported in the literature (Feng and Thompson, 1990; Newton et al., 1994; WHO, 1994), though some studies reported much shorter (3 days) or longer (2 years) half-lives in soil (WHO, 1994). Half-life of AMPA in soil was 46 days. The present study supports that the half-life (50 days) selected by Pesticide Research Institute for the prediction of the transport of glyphosate in broom-infested Mt. Tamalpais watershed is in good agreement with the field measured value. The observed half-life of glyphosate in soil implies that more than 50% of soil imbedded glyphosate would be degraded during the proposed application window (July 15 through September 15) if the application is made in early part of the application window. It is commonly known that pesticides aged in soil particles desorb much less than freshly applied pesticides (Alexander , 1995; Park et al., 2004; Regitano et al., 2006), indicating that aged glyphosate is less susceptible to wash-off by stormwater. Ratcliff et al. (2006) showed that glyphosate didn't cause any significant impacts on microbial community structure when glyphosate is applied at the recommended field rate (less than 5 kg/ha), which is much higher than the rate (less than 2 qt/ac = 1.14 kg/ac = 2.82 kg/ha) used in the present study.

Half-life in broom leaves that failed to drop to ground

Concentrations of glyphosate in broom leaves didn’t exhibit significant changes over the 84 days of study period for the both sites (Figure 4), indicating that half-life of glyphosate is likely to be much longer than 84 days as long as the leaves remain attached to the stems and branches. This slow degradation of glyphosate might be due to limited microbial activity on the leaves and time of year when plants are operating closer to dormancy. Photolysis also seemed to be insignificant. Large variations observed in glyphosate concentrations were presumably because the amount of glyphosate in each broom leaf was not homogeneous. As shown in Figure 2, many leaves were still attached to the stems and branches even 7 weeks after the application. Considering broom leaves were collected from multiple stems and branches randomly, glyphosate data indicates that broom leaves remained on the stems and branches still had herbicide mixture at levels similar to those found in early period samples. To calculate overall degradation of glyphosate, the slow degradation of glyphosate on leaves needs to be combined together with relatively faster degradation in soils. Because the present study didn't quantify what fraction of the leaves remained on the stems and branches during the study period, the overall half-life remains inconclusive.

The applied herbicide mixture dried within several hours of application so extra cautions are needed during this period to avoid any possible elevated exposure of humans to the applied mixture. Once the applied herbicide mixture dries, exposure of humans to the mixture through gentle brushing up against treated vegetation is expected to be substantially less than exposure to wet herbicide mixture. It is commonly known that glyphosate has a tendency to quickly penetrate into the internal structure of plant leaves (Gougler and Geiger, 1981; Feng et al., 1998, 1999). The fraction of the applied herbicide remained on the surface of the leaves likely declines over time, reducing the potential for dermal exposure. Although the present study was not designed to determine the extent of the exposure through dermal contact with the glyphosate treated vegetation, dermal contact is not likely a significant exposure route because glyphosate is poorly absorbed through the human skin. In a study using human autopsy samples, less than two percent of the applied glyphosate penetrated the skin when Roundup was applied in 1:20 to 1:32 dilutions to thigh skin (Wester et al., 1991). Glyphosate is quickly absorbed by leaves and shoots of weeds but does not penetrate woody stems of trees and animal skins.

Transport by surface stormwater runoff and soil infiltration

Glyphosate and AMPA were not found in both dissolved and particle phases of all stormwater runoff collected from the application site A. Roy et al. (1989) also reported no glyphosate in surface runoff samples. It is likely to be because glyphosate and AMPA tend to be strongly adsorbed to plant litters and soil particles that generally do not move by runoff especially in forested environments. No stormwater runoff samples were collected from the application site B because the site failed to produce run-off.

Glyphosate was detected in core soils up to 30 cm deep with the highest concentrations in the top layer (Figure 5). AMPA was not detected below 18 cm, which is presumably because AMPA is less water soluble than glyphosate. Other studies (Roy et al., 1989) also found similar depth profiles in forest core soils. Laboratory infiltration simulation exhibited that the glyphosate could penetrate deeper as water infiltration rate increases (Figure 6). But the water infiltration rate used for the laboratory study was not realistic. Considering relatively fast degradation of glyphosate in the surface soil and slower infiltration rates, this deeper infiltration is unlikely to happen under the real conditions

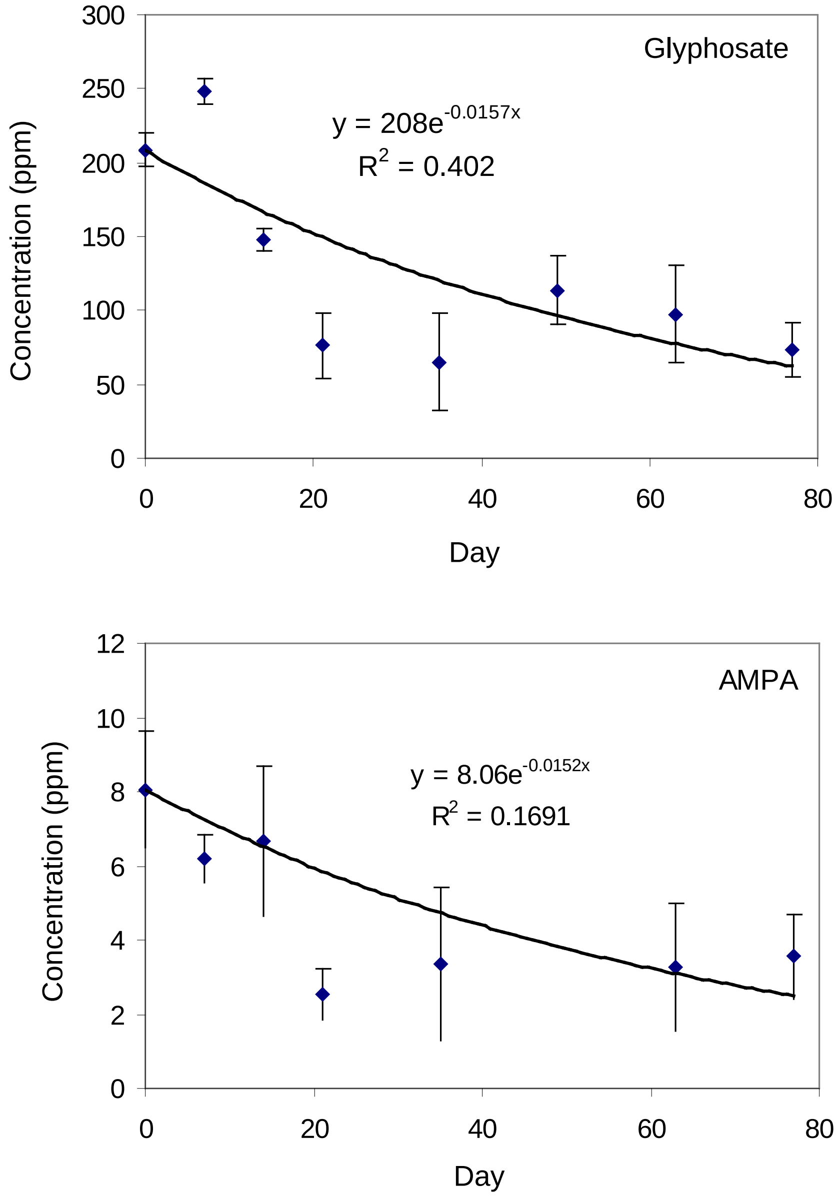


Figure 3. Changes of glyphosate and AMPA concentrations in the surface soils in Mt. Tamalpais.

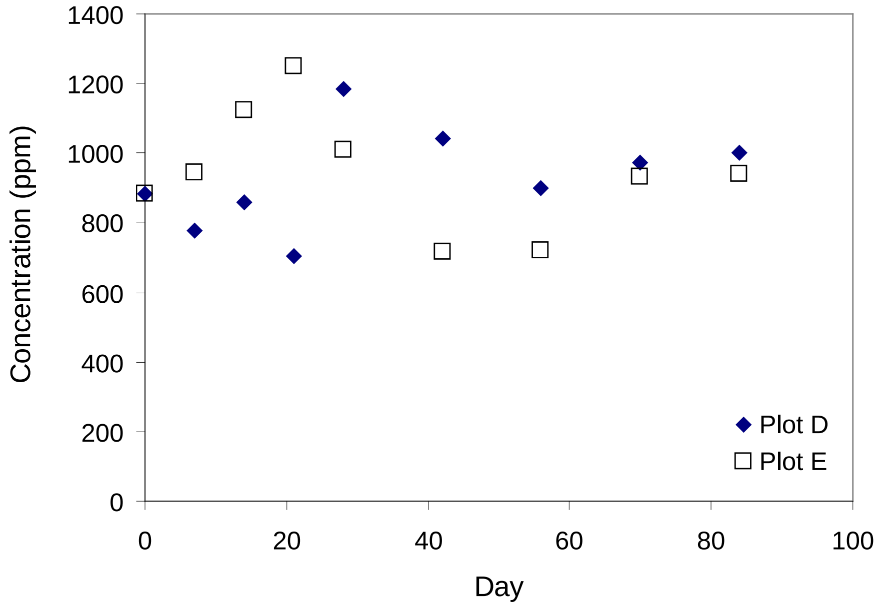


Figure 4. Changes in concentrations of glyphosate in broom leaves (still attached to plant) after the application of the herbicide mixture (Aquamater plus Competitor).

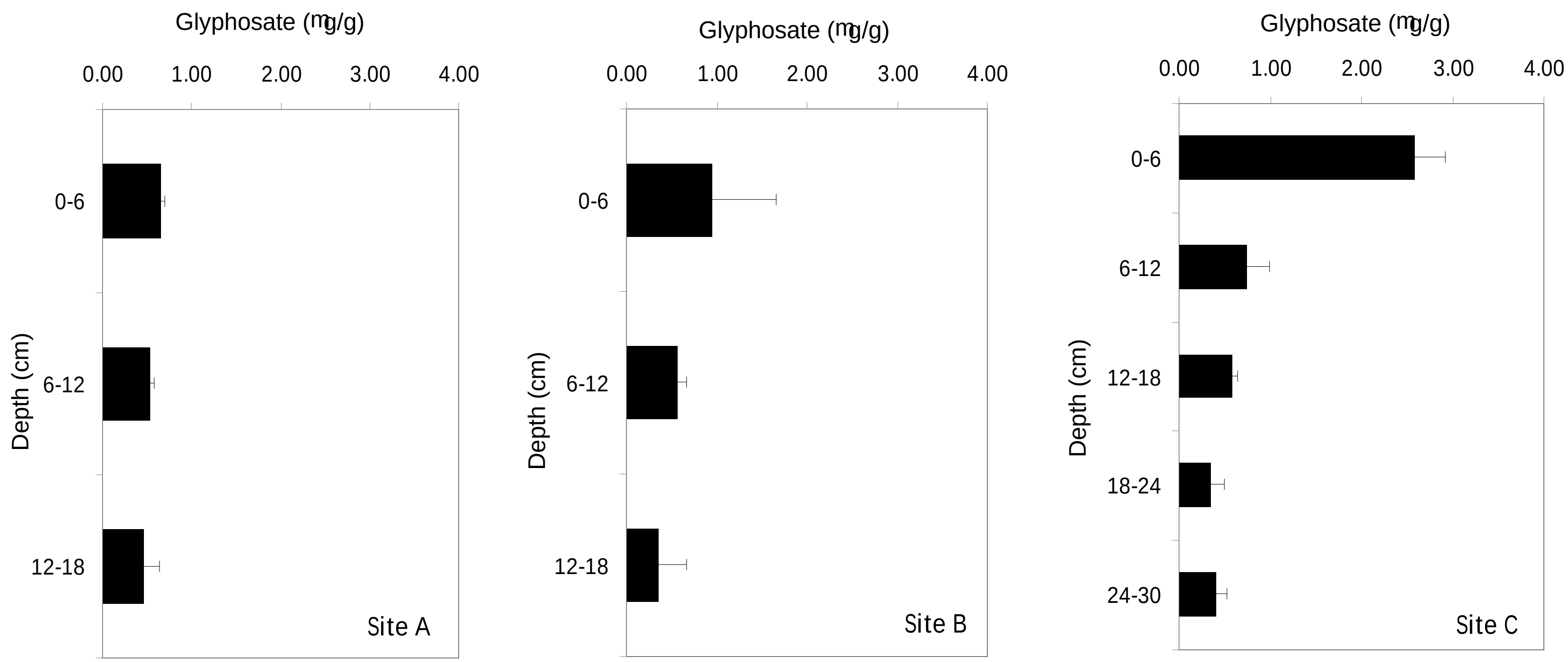


Figure 5. Glyphosate concentrations in core soils collected from the application sites A, B, and C in June 2010.

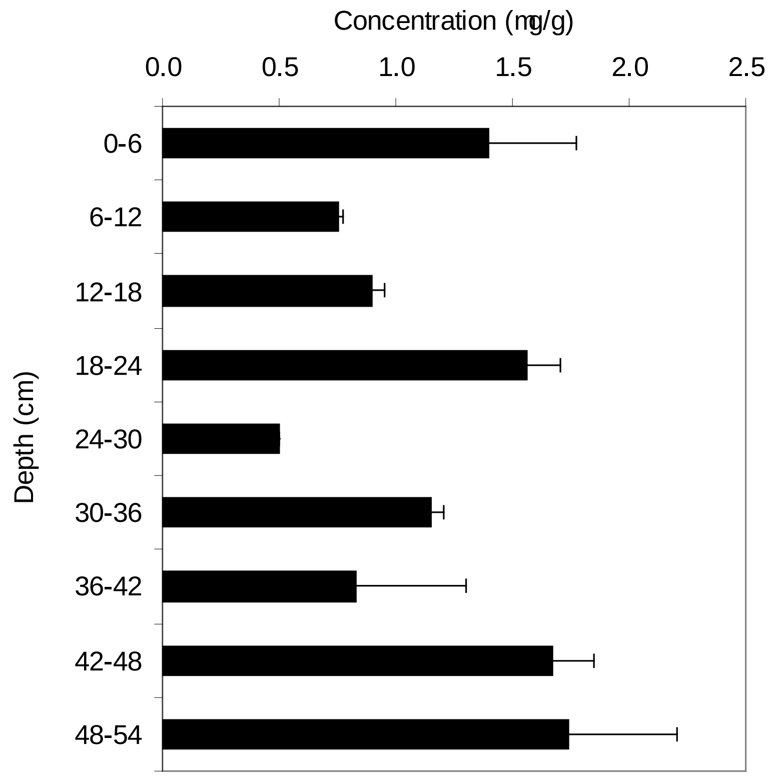


Figure 6. Glyphosate concentrations in core soil layers after the laboratory infiltration simulation. Glyphosate concentrations are averages of two soil cores collected from the application site C.

References

Alexander M. 1995. How toxic are toxic chemicals in soil? Environmental Science and Technology 29, 2713-2717.

Gougler JA, Geiger DB. 1981. Uptake and distribution of N-phosphonomethylglycine in sugar beet plants. Plant Physiology 68, 668-672.

Feng JC, Thompson DG. 1990. Fate of glyphosate in a Canadian forest watershed. 2. Persistence in foliage and soils. Journal of Agricultural and Food Chemistry 38, 1118-1125.

Feng PCC, Ryerse JS, Sammons RD. 1998. Correlation of leaf damage with uptake and translocation of glyphosate in velvetleaf (*Abutilon theophrasti*). Weed Technology 12, 300-307.

Feng PCC, Pratley JE, Bohn JA. 1999. Resistance to glyphosate in Lolium rigidum. II. Uptake, translocation, and metabolism. Weed Science 47, 412-415.

Huang X, Pedersen T, Fisher M, White R, Young TM. 2004. Herbicide runoff along highways 1. Field observation. Environmental Science and Technology 38, 3263-3271.

Lee EA, Strahan AP, Thurman EM. 2002. Determination of glyphosate, aminomethylphosphonic acid, and glufosinate in water using online solid-phase extraction and high-performance liquid chromatography/mass spectrometry. Open-File Report 01-454. U.S. Geological Survey, Lawrence, KS.

MMWD. 2008. Chemical weed control techniques. Interim background report No. 2. Marin Municipal Water District. Corte Madera, CA.

Newton M, Howard KM, Kelpsas BR, Danhaus R, Lottman CM, Dubelman S. 1984. Fate of glyphosate in an Oregon forest ecosystem. Journal of Agricultural and Food Chemistry 32, 1144-1151.

OEHHA. 2007. Public health goal for glyphosate in drinking water. Office of Environmental Health Hazard Assessment, California Environmental Protection Agency, Oakland, CA.

Park J-H, Feng Y, Cho SY, Voice TC, Boyd SA. 2004. Sorbed atrazine shifts into non-desorbable sites of soil organic matter during aging. Water Research 38, 3881-3892.

Ratcliff AW, Busse MD, Shestak CJ. 2006. Changes in microbial community structure following herbicide (glyphosate) additions to forest soils. Applied Soil Ecology 34, 114-124.

Regitano JB, Koskinen WC, Sadowsky MJ. 2006. Influence of soil aging on sorption and bioavailability of simazine. Journal of Agricultural and Food Chemistry.

Roy DN, Konar SK, Banerjee S, Charles DA, Thompson DG, Prasad R. 1989. Persistence, movement, and degradation of glyphosate in selected Canadian Boreal forest soils. Environmental Science and Technology 37, 437-440.

U.S. EPA. 1993. Re-registration eligibility decision (RED) document for glyphosate. EPA-738-F-93-011, U.S. Environmental Protection Agency, Washington, DC.

Wester RC, Melendres J, Sarason R, McMaster J, Maibach HI. 1991. Glyphosate skin binding, absorption, residual tissue distribution, and skin decontamination. Fundamental and Applied Toxicology 16, 725-732.

WHO. 1994. Glyphosate. Environmental Health Criteria, NO. 159. World Health Organization, Geneva, Switzerland.

GLYPHOSATE

1. Exposure Data

1.1 Identification of the agent

1.1.1 Nomenclature

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

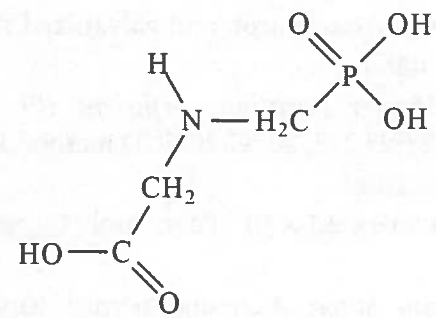
Chem. Abstr. Serv. Name: *N*-(phosphonomethyl)glycine

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

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

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

1.1.2 Structural and molecular formulae and relative molecular mass



Molecular formula: $C_3H_8NO_5P$
Relative molecular mass: 169.07

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

1.1.3 Chemical and physical properties of the pure substance

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

Solubility: The acid is of medium solubility at 11.6 g/L in water (at 25 °C) and insoluble in common organic solvents such as acetone, ethanol, and xylene; the alkali-metal and amine salts are readily soluble in water (Tomlin, 2000).

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

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

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

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

Henry's law: $< 2.1 \times 10^{-7}$ Pa m³ mol⁻¹ (Tomlin, 2000).

Conversion factor: Assuming normal temperature (25 °C) and pressure (101 kPa), mg/m³ = 6.92 × ppm.

1.1.4 Technical products and impurities

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

1.2 Production and use

1.2.1 Production

(a) Manufacturing processes

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

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

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

There are reportedly more than 750 products containing glyphosate for sale in the USA alone (NPIC, 2010). Formulated products contain various non-ionic surfactants, most notably polyethyloxylated tallowamine (POEA), to facilitate uptake by plants (Székács & Darvas, 2012). Formulations might contain other active ingredients, such as simasine, 2,4-dichlorophenoxyacetic acid (2,4-D), or 4-chloro-2-methylphenoxyacetic acid (IPCS, 1996), with herbicide resistance driving demand for new herbicide formulations containing multiple active ingredients (Freedonia, 2012).

(b) Production volume

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

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

1.2.2 Uses

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

(a) Agriculture

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

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

(b) Residential use

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

(c) Other uses

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

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

(d) Regulation

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

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

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

1.3 Measurement and analysis

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

Table 1.1 Methods for the analysis of glyphosate

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

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

1.4 Occurrence and exposure

1.4.1 Exposure

(a) Occupational exposure

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

(b) Community exposure

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

Table 1.2 Occupational and para-occupational exposure to glyphosate

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

Table 1.2 (continued)

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

LOD, limit of detection; ND, not detected; NR, not reported on the environmental occurrence of glyphosate (Kolpin *et al.*, 2006).

(i) Air

Very few studies of glyphosate in air were available to the Working Group. Air and rain-water samples were collected during two growing seasons in agricultural areas in Indiana, Mississippi, and Iowa, USA (Chang *et al.*, 2011). The frequency of glyphosate detection ranged from 60% to 100% in air and rain samples, and concentrations ranged from < 0.01 to 9.1 ng/m³ in air samples and from < 0.1 to 2.5 μg/L in rainwater samples. Atmospheric deposition was measured at three sites in Alberta, Canada. Rainfall and particulate matter were collected as total deposition at 7-day intervals throughout the growing season. Glyphosate deposition rates ranged from < 0.01 to 1.51 μg/m² per day (Humphries *et al.*, 2005).

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

(ii) Water

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

(iii) Residues in food and dietary intake

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

(iv) Household exposure

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

(v) Biological markers

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

1.4.2 Exposure assessment

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

Table 1.3 Concentration of glyphosate and AMPA in water

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

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

Table 1.4 Concentrations of glyphosate in food

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

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

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

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

2. Cancer in Humans

2.0 General discussion of epidemiological studies

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

2.1 Cohort studies

See Table 2.1

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

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

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

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

Table 2.1 Cohort studies of cancer and exposure to glyphosate

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

Table 2.1 (continued)

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

Table 2.1 (continued)

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

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

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

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

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

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

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

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

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

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

2.2.1 Non-Hodgkin lymphoma

See Table 2.2

(a) Case–control studies in the midwest USA

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

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

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

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

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

Table 2.2 (continued)

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

Table 2.2 (continued)

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

Table 2.2 (continued)

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

Table 2.2 (continued)

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

Table 2.2 (continued)

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

Table 2.2 (continued)

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

Table 2.2 (continued)

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

Table 2.2 (continued)

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

ALL, acute lymphocytic leukaemia; B-CLL, chronic lymphocytic leukaemia; CLL, chronic lymphocytic leukaemia; HCL, hairy cell leukaemia; HL, Hodgkin lymphoma; LPS, lymphoproliferative syndrome; MCPA, 2-methyl-4-chlorophenoxyacetic acid; MM, multiple myeloma; NHL, non-Hodgkin lymphoma; NR, not reported; ref., reference; STS, soft tissue sarcoma population-based, and conducted in farming areas. Potential confounding from multiple exposures was accounted for in the analysis.]

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

(b) The cross-Canada case–control study

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

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

(c) Case–control studies in Sweden

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

Hardell & Eriksson (1999) reported the results of a population-based case–control study on the incidence of NHL in men associated with pesticide exposure in four northern counties in Sweden. Exposure data was collected by questionnaire (also supplemented by telephone interviews) from 404 cases (192 deceased) and 741 controls (matched by age, sex, county, and vital status). Increased risks of NHL were found for subjects exposed to herbicides and fungicides. The odds ratio for ever-use of glyphosate was 2.3 (95% CI, 0.4–13; 4 exposed cases) in a univariate analysis, and 5.8 (95% CI, 0.6–54) in a multivariable analysis. [The exposure frequency was low for glyphosate, and the study had limited power to detect an effect. The variables included in the multivariate analysis were not specified. This study may have overlapped partially with those of Hardell *et al.* (2002).]

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

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

(d) Other case–control studies in Europe

Orsi *et al.* (2009) reported the results of a hospital-based case–control study conducted in six centres in France between 2000 and 2004. Incident cases with a diagnosis of lymphoid neoplasm aged 20–75 years and controls of the same age and sex as the cases were recruited in the same hospital, mainly in the orthopaedic and rheumatological departments during the same period. [The Working Group noted that the age of case eligibility was given in the publication as 20–75 years in the materials and methods section, but as 18–75 years in the abstract.] Exposures to pesticides were evaluated through specific interviews and case-by-case expert reviews. The analyses included 491 cases (244 cases of NHL, 87 cases of Hodgkin lymphoma), 104 of lymphoproliferative syndrome, and 56 cases of multiple myeloma), and 456 age- and sex-matched controls. Positive associations between some subtypes and occupational exposure to several pesticides were noted. The odds ratios associated with any exposure to glyphosate were 1.2 (95% CI, 0.6–2.1; 27 exposed cases) for all lymphoid neoplasms combined, 1.0 (95% CI, 0.5–2.2; 12 exposed cases) for NHL, 0.6 (95% CI, 0.2–2.1; 4 exposed cases) for lymphoproliferative syndrome, 2.4 (95% CI, 0.8–7.3) for multiple myeloma, and 1.7 (95% CI, 0.6–5.0; 6 exposed cases) for Hodgkin lymphoma, after adjusting for age, centre, and socioeconomic category ("blue/white collar").

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

2.2.2 Other haematopoietic cancers

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

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

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

2.3 Case–control studies on other cancer sites

2.3.1 Cancer of the oesophagus and stomach

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

2.3.2 Cancer of the brain

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

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

Lee *et al.* (2005) evaluated the association between farming and agricultural pesticide use and risk of adult glioma in a population-based case–control study in eastern Nebraska, USA. Cases of glioma were in men and women (n = 251) and were compared with population controls from a previous study (n = 498). A telephone interview was conducted for 89% of the cases and 83% of the controls. Adjusted odds ratios for farming and for use of individual and chemical classes of insecticides and herbicides were calculated using non-farmers as the reference category. Among men, ever living or working on a farm and duration of farming were associated with significantly increased risks of glioma, but the positive findings were limited to proxy respondents. Among women, there were no positive associations with farming activities among self or proxy respondents. Some specific pesticide families and individual pesticides were associated with significantly increased risks among male farmers, but most of the positive associations were limited to proxy respondents. There was a non-significant excess risk with glyphosate use for the overall group (OR, 1.5; 95% CI, 0.7–3.1; 17 exposed cases), but there was inconsistency between observations for self-respondents (OR, 0.4; 95% CI, 0.1–1.6) and observations for proxy respondents (OR, 3.1; 95% CI, 1.2–8.2). [The study had limited power to detect an effect of glyphosate use, and the inconsistencies for self and proxy respondents made the results difficult to interpret.]

2.3.3 Soft tissue sarcoma

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

2.3.4 Cancer of the prostate

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

2.3.5 Childhood cancer

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

2.4. Meta-analyses

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

3. Cancer in Experimental Animals

3.1 Mouse

See Table 3.1

3.1.1 Dietary administration

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

Table 3.1 Studies of carcinogenicity with glyphosate in mice

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

Table 3.1 (continued)

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

bw, body weight; DMBA, 7,12-dimethylbenz[a]anthracene; EPA, United States Environmental Protection Agency; F, female; M, male; mo, month; NR, not reported; NS, not significant; POEA, polyethoxylated tallowamine; PWG, pathology working group; TPA, 12-O-tetradecanoyl-phorbol-13-acetate; vs, versus; wk, week; yr, year were provided for female mice. No other tumour sites were identified (EPA, 1985a). Subsequent to its initial report (EPA, 1985a), the United States Environmental Protection Agency (EPA) recommended that additional renal sections be cut and evaluated from all male mice in the control and treated groups. The pathology report for these additional sections (EPA, 1985b) indicated the same incidence of renal tubule adenoma as originally reported, with no significant increase in incidence between the control group and treated groups by pairwise comparison. However, as already reported above, the test for linear trend in proportions resulted in a significance of $P = 0.016$. The EPA (1986) also requested that a pathology working group (PWG) be convened to evaluate the tumours of the kidney observed in male mice treated with glyphosate, including the additional renal sections. In this second evaluation, the PWG reported that the incidence of adenoma of the renal tubule was 1/49 (2%), 0/49, 0/50, 1/50 (2%) [not statistically significant]; the incidence of carcinoma of the renal tubule was 0/49, 0/49, 1/50 (2%), 2/50 (4%) [$P = 0.037$, trend test for carcinoma]; and the incidence of adenoma or carcinoma (combined) of the renal tubule was 1/49 (2%), 0/49, 1/50 (2%), 3/50 (6%) [$P = 0.034$, trend test for combined]. [The Working Group considered that this second evaluation indicated a significant increase in the incidence of rare tumours, with a dose-related trend, which could be attributed to glyphosate. Chandra & Frith (1994) reported that only 1 out of 725 [0.14%] CD-1 male mice in their historical database had developed renal cell tumours (one carcinoma).]

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

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

3.1.2 Initiation–promotion

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

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

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

3.1.3 Review articles

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

In the first study (identified as Study 12, 1997a), groups of 50 male and 50 female CD-1 mice [age at start not reported] were given diets containing glyphosate (purity, 94–96%) at a concentration of 0, 1600, 8000, or 40 000 ppm for 18 months. The increase in the incidence of bronchiolo-alveolar adenoma and carcinoma, and of lymphoma, was reported to be not statistically significant in males and females receiving glyphosate. [The Working Group was unable to evaluate this study because of the limited experimental data provided in the review article and supplemental information.]

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

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

3.2 Rat

See Table 3.2

3.2.1 Drinking-water

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

In another study with drinking-water, Chruscielska *et al.* (2000) gave groups of 55 male and 55 female Wistar rats (age, 6–7 weeks) drinking-water containing an ammonium salt of glyphosate as a 13.85% solution [purity of glyphosate, not reported] that was used to make aqueous solutions of 0 (control), 300, 900, and 2700 mg/L, for 24 months [details on the dosing regimen were not reported]. The authors reported that survival and body-weight gain were similar in treated and control animals. No significant increase in tumour incidence was reported in any of the treated groups. [The Working Group noted the limited information provided on dosing regimen, histopathological examination method, and tumour incidences.]

3.2.2 Dietary administration

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

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

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

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

Table 3.2 Studies of carcinogenicity with glyphosate in rats

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

Table 3.2 (continued)

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

Table 3.2 (continued)

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

bw, body weight; d, day; F, female; M, male; mo, month; NR, not reported; NS, not significant; wk, week; yr, year males ($P = 0.016$) and of thyroid follicular cell adenoma in females ($P = 0.031$). [The Working Group noted that there was no apparent progression to carcinoma for either tumour type.]

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

3.2.3 Review articles

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

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

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

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

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

4. Mechanistic and Other Relevant Data

4.1 Toxicokinetic data

4.1.1 Introduction

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

4.1.2 Absorption

(a) Humans

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

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

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

Small amounts of glyphosate can be absorbed after dermal exposures in humans in vitro. For example, when an aqueous solution of 1% glyphosate was applied in an in-vitro human skin model, only 1.4% of the applied dose was absorbed through the skin. Glyphosate is typically formulated as an isopropylamine salt, and is dissolved in a water-based vehicle, while the stratum corneum is a lipid-rich tissue (Wester *et al.*, 1991). In-vitro studies using human skin showed that percutaneous absorption of a glyphosate-based formulation was no more than 2% of the administered dose over a concentration range of 0.5–154 μg/cm² and a topical volume range of 0.014–0.14 mL/cm². In addition, very little glyphosate (≤ 0.05% of the administered dose) was sequestered in the stratum corneum after dermal application (Wester *et al.*, 1991).

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

(b) Experimental systems

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

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

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

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

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

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

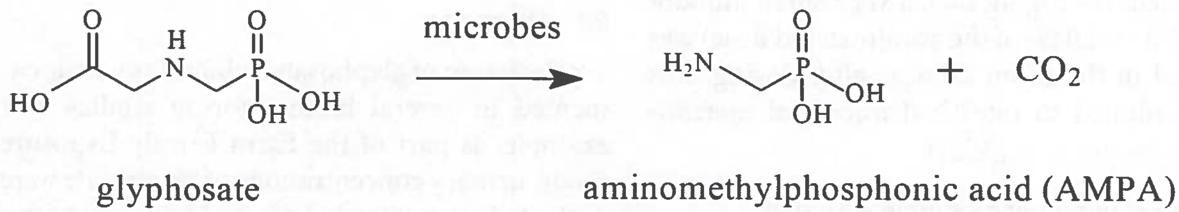
4.1.3 Distribution

(a) Humans

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

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

Fig. 4.1 Microbial metabolism of glyphosate to AMPA



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

(b) Experimental systems

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

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

4.1.4 Metabolism and modulation of metabolic enzymes

(a) Metabolism

Glyphosate is degraded in the environment by soil microbes, primarily to AMPA and carbon dioxide (Fig. 4.1; Jacob *et al.*, 1988). A minor pathway for the degradation of glyphosate in bacteria (*Pseudomonas sp.* strain LBr) is via conversion to glycine (Jacob *et al.*, 1988). In a case of deliberate poisoning with a glyphosate-based formulation, small amounts of AMPA (15.1 μg/mL) were detectable in the blood (Motojyuku *et al.*, 2008) [suggesting that this pathway might also operate in humans]. In rats given a single high oral dose of glyphosate (100 mg/kg bw), small amounts of AMPA were detected in the plasma (Bernal *et al.*, 2010). In male Sprague-Dawley rats given an oral dose of glyphosate (10 mg/kg bw), a very small amount of AMPA (< 0.04% of the administered dose) was detected in the colon 2 hours after dosing; this was attributed to intestinal microbial metabolism (Brewster *et al.*, 1991).

(b) Modulation of metabolic enzymes

(i) Humans

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

(ii) Experimental systems

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

4.1.5 Excretion

(a) Humans

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

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

(b) Experimental systems

In an NTP study in Fisher 344 rats given a single oral dose of [^{14}C]-labelled glyphosate (5.6 or 56 mg/kg bw), it was shown that > 90% of the radiolabel was eliminated in the urine and faeces within 72 hours (Chan & Mahler, 1992). In Sprague-Dawley rats given [^{14}C]-labelled glyphosate at an oral dose of 10 or 1000 mg/kg bw, ~60–70% of the administered dose was excreted in the faeces, and the remainder in the urine (EPA, 1993b). By either route, most (98%) of the administered dose was excreted as unchanged parent compound. AMPA was the only metabolite found in the urine (0.2–0.3% of the administered dose) and faeces (0.2–0.4% of the administered dose). [The large amount of glyphosate excreted in the faeces is consistent with its poor oral absorption.] Less than 0.3% of the administered dose was expired as carbon dioxide.

In rhesus monkeys given glyphosate as an intravenous dose (9 or 93 µg), > 95% of the administered dose was excreted in the urine (Wester *et al.*, 1991). Nearly all the administered dose was eliminated within 24 hours. In contrast, in rhesus monkeys given glyphosate by dermal application (5400 µg/20 cm²), only 2.2% of the administered dose was excreted in the urine within 7 days (Wester *et al.*, 1991).

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

4.2 Mechanisms of carcinogenesis

4.2.1 Genetic and related effects

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

(a) Humans

(i) Studies in exposed humans

See Table 4.1

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

Bolognesi *et al.* (2009) studied community residents (137 women of reproductive age and their 137 spouses) from five regions in Colombia. In three regions with exposures to glyphosate-based formulations from aerial spraying, blood samples were taken from the same individuals at three time-points (before spraying (baseline), 5 days after spraying and 4 months after spraying) to determine the frequency of micronucleus formation in lymphocytes. The baseline frequency of binucleated cells with micronuclei was significantly higher in subjects from the three regions where there had been aerial spraying with glyphosate-formulations and in a fourth region with pesticide exposure (but not through aerial spraying), compared with a reference region (without use of pesticide). The frequency of micronucleus formation in peripheral blood lymphocytes was significantly increased, compared with baseline levels in the same individuals, after aerial spraying with glyphosate-based formulations in each of the three regions (see Table 4.1; Bolognesi *et al.*, 2009). Immediately after spraying, subjects who reported direct contact with the glyphosate-based spray showed a higher frequency of binucleated cells with micronuclei. However, the increase in frequency of micronucleus formation observed immediately after spraying was not consistent with the rates of application used in the regions, and there was no association between self-reported direct contact with pesticide sprays and frequency of binucleated cells with micronuclei. In subjects from one but not other regions, the frequency of binucleated cells with micronuclei was significantly decreased 4 months after spraying, compared with immediately after spraying.

(ii) Human cells in vitro

See Table 4.2

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

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

(b) Experimental systems

(i) Non-human mammals in vivo

See Table 4.3

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

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

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

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

Table 4.1 Genetic and related effects of glyphosate in exposed humans

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

[a] +, positive; –, negative

NR, not reported; vs, versus

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

(ii) Non-human mammalian cells in vitro

See Table 4.4

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

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

(iii) Non-mammalian systems in vivo

See Table 4.5

Fish and other species

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

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

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

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

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

Table 4.2 (continued)

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

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

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

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

Insects

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

Plants

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

(iv) Non-mammalian systems in vitro

See Table 4.6

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

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

4.2.2 Receptor-mediated mechanisms

(a) Sex-hormone pathway disruption

(i) Humans

Studies in exposed humans

No data were available to the Working Group.

Human cells in vitro

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

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

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

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

Table 4.3 (continued)

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

Table 4.3 (continued)

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

[a] +, positive; -, negative; (+) or (-) positive/negative in a study with limited quality
bw, body weight; F, female; h, hour; HID, highest effective dose; i.p., intraperitoneal; LC, liquid chromatography; LED, lowest effective dose; M, male; PCE, polychromatic erythrocytes; p.o., oral; 8-OHdG, 8-hydroxydeoxyguanosine; UV, ultraviolet

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

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

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

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

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

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

Table 4.5 (continued)

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

Table 4.5 (continued)

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

Table 4.5 (continued)

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

Table 4.5 (continued)

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

Table 4.5 (continued)

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

Table 4.5 (continued)

Phylogenetic class	Species, strain, tissue	End-point	Test	Results[a]	Dose (LED or HID)	Comments	Reference
Caiman	*Caiman latirostris* (broad-snouted caiman), erythrocytes	Chromosomal damage	Micronucleus fomation	+	19.8 g/L	Glyphosate, 66.2% One dose tested; in-ovo exposure First spraying exposure at the beginning of incubation period, a second exposure on day 35, then incubation until hatching. Micronucleus formation, $P < 0.001$ Damage index, $P < 0.001$	Poletta *et al.* (2011)
Frog tadpole	*Rana catesbeiana* (ouaouaron), blood	DNA damage	DNA strand breaks, comet assay	+	1.687 mg/L, p.o.	Time of exposure, 24 h $P < 0.05$, with 6.75 mg/L; and $P < 0.001$ with 27 mg/L (with 108 mg/L, all died within 24 h)	Clements *et al.* (1997)
Frog	*Eleutherodactylus johnstonei* (Antilles coqui), erythrocytes	DNA damage	DNA strand breaks, comet assay	+	0.5 µg a.e./cm²	Glyphosate-based formulation, 480 g/L Exposure to an homogenate mist in a 300 cm² glass terrarium Time of exposure: 0.5, 1, 2, 4, 8 and 24 h $P < 0.05$	Meza-Joya *et al.* (2013)
Frog	*Euflictis cyanophlyctis* (Indian skittering frog), erythrocytes	Chromosomal damage	Micronucleus formation	+	1 mg a.e./L	Glyphosate isopropylamine salt, 41% Time of exposure: 24, 48, 72, and 96 h $P < 0.001$ at 24, 48, 72 and 96 h	Yadav *et al.* (2013)
Snail	*Biomphalaria alexandrina*, haemolymph	DNA damage	DNA strand breaks, comet assay	+	10 mg/L	Glyphosate, 48% Single dose tested only, for 24 h. The percentage of damaged DNA was 21% vs 4% (control) No statistical analysis	Mohamed (2011)
Oyster	Oysters, spermatozoa	DNA damage	DNA strand breaks, comet assay	–	5 µg/L	Glyphosate, 200 µg equivalent/L Time of exposure, 1 h	Akcha *et al.* (2012)

Table 4.5 (continued)

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

Table 4.5 (continued)

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

[a] +, positive; –, negative; (+) or (–) positive/negative in a study with limited quality
a.e., acid equivalent; AMPA, aminomethyl phosphonic acid; bw, body weight; ENA, erythrocytic nuclear abnormalities; Endo III, endonuclease III; FPG, formamidopyrimidine glycosylase; h, hour; HID, highest ineffective dose; LC_{50}, median lethal dose; LED, lowest effective dose; NOEC, no-observed effect concentration; p.o., oral; SMART, somatic mutation and recombination test

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

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

Table 4.6 (continued)

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

[a] +, positive; –, negative; (+) or (–) positive/negative in a study with limited quality
FADU, fluorometric analysis of DNA unwinding; HIC, highest ineffective concentration; LEC, lowest effective concentration; NR, not reported; NT, not tested; UVB, ultraviolet B

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

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

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

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

(ii) Non-human mammalian experimental systems

In vivo

No data were available to the Working Group.

In vitro

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

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

(iii) Non-mammalian experimental systems

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

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

(b) Other pathways

(i) Humans

Studies in exposed humans

No data were available to the Working Group.

Human cells in vitro

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

(ii) Non-human mammalian experimental systems

In vivo

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

In vitro

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

(iii) Non-mammalian experimental systems

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

4.2.3 Oxidative stress, inflammation, and immunosuppression

(a) Oxidative stress

(i) Humans

Studies in exposed humans

No data were available to the Working Group.

Human cells in vitro

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

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

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

(ii) Non-human mammalian experimental systems

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

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

(iii) Non-mammalian experimental systems

Positive associations between exposure to glyphosate and oxidative stress were reported in various tissues in aquatic organisms (reviewed in Slaninova *et al.*, 2009). Glyphosate and various glyphosate-based formulations have been tested in various fish species for effects on a plethora of end-points (e.g. lipid peroxidation, DNA damage, expression of antioxidant enzymes, levels of glutathione), consistently presenting evidence that glyphosate can cause oxidative stress in fish (Lushchak *et al.*, 2009; Ferreira *et al.*, 2010; Guilherme *et al.*, 2010, 2012a, b, 2014a, b; Modesto & Martinez, 2010a, b; Cattaneo *et al.*, 2011; Glusczak *et al.*, 2011; de Menezes *et al.*, 2011; Ortiz-Ordoñez *et al.*, 2011; Nwani *et al.*, 2013; Marques *et al.*, 2014, 2015; Sinhorin *et al.*, 2014; Uren Webster *et al.*, 2014). Similar effects were observed in bullfrog tadpoles exposed to a glyphosate-based formulation (Costa *et al.*, 2008), and in the Pacific oyster exposed to a pesticide mixture containing glyphosate (Geret *et al.*, 2013).

(b) Inflammation and immunomodulation

(i) Humans

Studies in exposed humans

No data were available to the Working Group.

Human cells in vitro

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

(ii) Non-human mammalian experimental systems

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

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

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

(iii) Non-mammalian experimental systems

A positive association between exposure to glyphosate and immunotoxicity in fish has been reported. Kreutz *et al.* (2011) reported alterations in haematological and immune-system parameters in silver catfish (*Rhamdia quelen*) exposed to sublethal concentrations (10% of the median lethal dose, LC_{50}, at 96 hours) of a glyphosate-based herbicide. Numbers of blood erythrocytes, thrombocytes, lymphocytes, and total leukocytes were significantly reduced after 96 hours of exposure, while the number of immature circulating cells was increased. The phagocytic index, serum bacteria agglutination, and total peroxidase activity were significantly reduced after 24 hours of exposure. Significant decreases in serum bacteria agglutination and lysozyme activity were found after 10 days of exposure. No effect on serum bactericidal and complement natural haemolytic activity was seen after 24 hours or 10 days of exposure to glyphosate.

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

4.2.4 Cell proliferation and death

(a) Humans

(i) Studies in exposed humans

No data were available to the Working Group.

(ii) Human cells in vitro

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

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

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

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

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

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

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

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

(b) Non-human mammalian experimental systems

(i) In vivo

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

(ii) In vitro

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

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

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

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

4.2.5 Other mechanisms

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

4.3 Data relevant to comparisons across agents and end-points

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

4.4 Cancer susceptibility data

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

4.5 Other adverse effects

4.5.1 Humans

In the USA in the past decade, poison-control centres have reported more than 4000 exposures to glyphosate-containing herbicides, of which several hundred were evaluated in a health-care facility, and fatalities were rare (Rumack, 2015). In a pesticide surveillance study carried out by the National Poisons Information Service of the United Kingdom, glyphosate was among the most common pesticide exposure implicated in severe or fatal poisoning cases between 2004 and 2013 (Perry *et al.*, 2014). Deliberate poisonings with glyphosate resulting in toxicity and fatality have been reported in many countries, including Australia (Stella & Ryan, 2004), Denmark (Mortensen *et al.*, 2000), India (Mahendrakar *et al.*, 2014), Japan (Motojyuku *et al.*, 2008), Republic of Korea (Park *et al.*, 2013), New Zealand (Temple & Smith, 1992), Sri Lanka (Roberts *et al.*, 2010), Taiwan, China (Chen *et al.*, 2009), and Thailand (Sribanditmongkol *et al.*, 2012).

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

4.5.2 Experimental systems

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

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

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

Developmental and reproductive toxicity

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

5. Summary of Data Reported

5.1 Exposure data

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

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

5.2 Human carcinogenicity data

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

5.2.1 NHL and other haematopoietic cancers

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

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

In summary, case–control studies in the USA, Canada, and Sweden reported increased risks for NHL associated with exposure to glyphosate. The increased risk persisted in the studies that adjusted for exposure to other pesticides. The AHS cohort did not show an excess of NHL. The Working Group noted that there were excesses reported for multiple myeloma in three studies; however, they did not weight this evidence as strongly as that of NHL because of the possibility that chance could not be excluded; none of the risk estimates were statistically significant nor were they adjusted for other pesticide exposures.

5.2.2. Other cancer sites

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

5.3 Animal carcinogenicity data

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

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

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

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

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

5.4. Other relevant data

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

Glyphosate was measured in human blood. No data on parenchymal tissue distribution for glyphosate in humans were available to the Working Group. In rats given glyphosate by oral administration, concentrations in tissues had the following rank order: kidneys > spleen > fat > liver. Repeated administration had no effect on the distribution of glyphosate. In a study in rats, the half-life of glyphosate in plasma was estimated to be more than 1 day, indicating that glyphosate is not rapidly eliminated.

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

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

Glyphosate is not electrophilic.

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

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

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

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

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

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

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

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

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

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

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

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

6. Evaluation

6.1 Cancer in humans

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

6.2 Cancer in experimental animals

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

6.3 Overall evaluation

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

6.4 Rationale

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

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

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

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

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

References

Abraxis (2005). Glyphosate Plate Kit Part No. 500086. Warminster (PA): Abraxis, LLC. Available from: http://www.abraxiskits.com/uploads/products/docfiles/184_PN500086USER.pdf, accessed 28 July 2015.

Acquavella JF, Alexander BH, Mandel JS, Gustin C, Baker B, Chapman P *et al.* (2004). Glyphosate biomonitoring for farmers and their families: results from the Farm Family Exposure Study. *Environ Health Perspect*, 112(3):321–6. doi:10.1289/ehp.6667 PMID:14998747

Akcha F, Spagnol C, Rouxel J (2012). Genotoxicity of diuron and glyphosate in oyster spermatozoa and embryos. *Aquat Toxicol*, 106–107:104–13. doi:10.1016/j.aquatox.2011.10.018 PMID:22115909

Alavanja MC, Samanic C, Dosemeci M, Lubin J, Tarone R, Lynch CF *et al.* (2003). Use of agricultural pesticides and prostate cancer risk in the Agricultural Health Study cohort. *Am J Epidemiol*, 157(9):800–14. doi:10.1093/aje/kwg040 PMID:12727674

Alavanja MC, Sandler DP, McMaster SB, Zahm SH, McDonnell CJ, Lynch CF *et al.* (1996). The Agricultural Health Study. *Environ Health Perspect*, 104(4):362–9. doi:10.1289/ehp.96104362 PMID:8732939

Alvarez-Moya C, Silva MR, Arámbula AR, Sandoval AI, Vasquez HC, González Montes RM (2011). Evaluation of genetic damage induced by glyphosate isopropylamine salt using *Tradescantia* bioassays. *Genet Mol Biol*, 34(1):127–30. doi:10.1590/S1415-47572010005000108 PMID:21637555

Alvarez-Moya C, Silva MR, Ramírez CV, Gallardo DG, Sánchez RL, Aguirre AC *et al.* (2014). Comparison of the *in vivo* and *in vitro* genotoxicity of glyphosate isopropylamine salt in three different organisms. *Genet Mol Biol*, 37(1):105–10. doi:10.1590/S1415-47572014000100016 PMID:24688297

Andreotti G, Freeman LE, Hou L, Coble J, Rusiecki J, Hoppin JA *et al.* (2009). Agricultural pesticide use and pancreatic cancer risk in the Agricultural Health Study Cohort. *Int J Cancer*, 124(10):2495–500. doi:10.1002/ijc.24185 PMID:19142867

Aris A, Leblanc S (2011). Maternal and fetal exposure to pesticides associated to genetically modified foods in Eastern Townships of Quebec, Canada. *Reprod Toxicol*, 31(4):528–33. doi:10.1016/j.reprotox.2011.02.004 PMID:21338670

Astiz M, de Alaniz MJ, Marra CA (2009a). Effect of pesticides on cell survival in liver and brain rat tissues. *Ecotoxicol Environ Saf*, 72(7):2025–32. doi:10.1016/j.ecoenv.2009.05.001 PMID:19493570

Astiz M, de Alaniz MJ, Marra CA (2009b). Antioxidant defense system in rats simultaneously intoxicated with agrochemicals. *Environ Toxicol Pharmacol*, 28(3):465–73. doi:10.1016/j.etap.2009.07.009 PMID:21784044

Astiz M, Hurtado de Catalfo GE, García MN, Galletti SM, Errecalde AL, de Alaniz MJ *et al.* (2013). Pesticide-induced decrease in rat testicular steroidogenesis is differentially prevented by lipoate and tocopherol. *Ecotoxicol Environ Saf*, 91:129–38. doi:10.1016/j.ecoenv.2013.01.022 PMID:23465731

Band PR, Abanto Z, Bert J, Lang B, Fang R, Gallagher RP *et al.* (2011). Prostate cancer risk and exposure to pesticides in British Columbia farmers. *Prostate*, 71(2):168–83. doi:10.1002/pros.21232 PMID:20799287

Battaglin WA, Kolpin DW, Scribner EA, Kuivila KM, Sandstrom MW (2005). Glyphosate, other herbicides, and transformation products in midwestern streams, 20021. *J Am Water Resour Assoc*, 41(2):323–32. doi:10.1111/j.1752-1688.2005.tb03738.x

Benachour N, Séralini GE (2009). Glyphosate formulations induce apoptosis and necrosis in human umbilical, embryonic, and placental cells. *Chem Res Toxicol*, 22(1):97–105. doi:10.1021/tx800218n PMID:19105591

Benachour N, Sipahutar H, Moslemi S, Gasnier C, Travert C, Séralini GE (2007). Time- and dose-dependent effects of Roundup on human embryonic and placental cells. *Arch Environ Contam Toxicol*, 53(1):126–33. doi:10.1007/s00244-006-0154-8 PMID:17486286

Bernal J, Bernal JL, Martin MT, Nozal MJ, Anadón A, Martínez-Larrañaga MR *et al.* (2010). Development and validation of a liquid chromatography-fluorescence-mass spectrometry method to measure glyphosate and aminomethylphosphonic acid in rat plasma. *J Chromatogr B Analyt Technol Biomed Life*

Sci, 878(31):3290–6. doi:10.1016/j.jchromb.2010.10.013 PMID:21106459

Blair A, Thomas K, Coble J, Sandler DP, Hines CJ, Lynch CF *et al.* (2011). Impact of pesticide exposure misclassification on estimates of relative risks in the Agricultural Health Study. *Occup Environ Med*, 68(7):537–41. doi:10.1136/oem.2010.059469 PMID:21257983

Blakley BR (1997). Effect of Roundup and Tordon 202C herbicides on antibody production in mice. *Vet Hum Toxicol*, 39(4):204–6. PMID:9251167

Bolognesi C, Bonatti S, Degan P, Gallerani E, Peluso M, Rabboni R *et al.* (1997). Genotoxic activity of glyphosate and its technical formulation Roundup. *J Agric Food Chem*, 45(5):1957–62. doi:10.1021/jf9606518

Bolognesi C, Carrasquilla G, Volpi S, Solomon KR, Marshall EJ (2009). Biomonitoring of genotoxic risk in agricultural workers from five Colombian regions: association to occupational exposure to glyphosate. *J Toxicol Environ Health A*, 72(15–16):986–97. doi:10.1080/15287390902929741 PMID:19672767

Bonini MG, Rota C, Tomasi A, Mason RP (2006). The oxidation of 2′,7′-dichlorofluorescein to reactive oxygen species: a self-fulfilling prophesy? *Free Radic Biol Med*, 40(6):968–75. doi:10.1016/j.freeradbiomed.2005.10.042 PMID:16540392

Borggaard OK, Gimsing AL (2008). Fate of glyphosate in soil and the possibility of leaching to ground and surface waters: a review. *Pest Manag Sci*, 64(4):441–56. doi:10.1002/ps.1512 PMID:18161065

Botero-Coy AM, Ibáñez M, Sancho JV, Hernández F (2013). Improvements in the analytical methodology for the residue determination of the herbicide glyphosate in soils by liquid chromatography coupled to mass spectrometry. *J Chromatogr A*, 1292:132–41. doi:10.1016/j.chroma.2012.12.007 PMID:23332301

Botero-Coy AM, Ibáñez M, Sancho JV, Hernández F (2013b). Direct liquid chromatography-tandem mass spectrometry determination of underivatized glyphosate in rice, maize and soybean. *J Chromatogr A*, 1313:157–65. doi:10.1016/j.chroma.2013.07.037 PMID:23891211

Brewster DW, Warren J, Hopkins WE 2nd (1991). Metabolism of glyphosate in Sprague-Dawley rats: tissue distribution, identification, and quantitation of glyphosate-derived materials following a single oral dose. *Fundam Appl Toxicol*, 17(1):43–51. doi:10.1016/0272-0590(91)90237-X PMID:1916078

Brown LM, Blair A, Gibson R, Everett GD, Cantor KP, Schuman LM *et al.* (1990). Pesticide exposures and other agricultural risk factors for leukemia among men in Iowa and Minnesota. *Cancer Res*, 50(20):6585–91. PMID:2208120

Brown LM, Burmeister LF, Everett GD, Blair A (1993). Pesticide exposures and multiple myeloma in Iowa men. *Cancer Causes Control*, 4(2):153–6. doi:10.1007/BF00053156 PMID:8481493

Brüch W, Rosenborg AE, Johler RK, Gudmunsson L, Nielsen CB, Plauborg F, et al. (2013). Monitoring results 1999–2012. The Danish Pesticide Leaching Assessment Programme. Available from: http://pesticidvarsling.dk/publ_result/index.html, accessed 1 December 2014.

Cantor KP, Blair A, Everett G, Gibson R, Burmeister LF, Brown LM *et al.* (1992). Pesticides and other agricultural risk factors for non-Hodgkin's lymphoma among men in Iowa and Minnesota. *Cancer Res*, 52(9):2447–55. PMID:1568215

Carreón T, Butler MA, Ruder AM, Waters MA, Davis-King KE, Calvert GM *et al.*; Brain Cancer Collaborative Study Group(2005). Gliomas and farm pesticide exposure in women: the Upper Midwest Health Study. *Environ Health Perspect*, 113(5):546–51. doi:10.1289/ehp.7456 PMID:15866761

Cattaneo R, Clasen B, Loro VL, de Menezes CC, Pretto A, Baldisserotto B *et al.* (2011). Toxicological responses of *Cyprinus carpio* exposed to a commercial formulation containing glyphosate. *Bull Environ Contam Toxicol*, 87(6):597–602. doi:10.1007/s00128-011-0396-7 PMID:21931962

Cattani D, de Liz Oliveira Cavalli VL, Heinz Rieg CE, Domingues JT, Dal-Cim T, Tasca CI *et al.* (2014). Mechanisms underlying the neurotoxicity induced by glyphosate-based herbicide in immature rat hippocampus: involvement of glutamate excitotoxicity. *Toxicology*, 320:34–45. doi:10.1016/j.tox.2014.03.001 PMID:24636977

Cavalcante DG, Martinez CB, Sofia SH (2008). Genotoxic effects of Roundup on the fish *Prochilodus lineatus*. *Mutat Res*, 655(1–2):41–6. doi:10.1016/j.mrgentox.2008.06.010 PMID:18638566

Cavaş T, Könen S (2007). Detection of cytogenetic and DNA damage in peripheral erythrocytes of goldfish (*Carassius auratus*) exposed to a glyphosate formulation using the micronucleus test and the comet assay. *Mutagenesis*, 22(4):263–8. doi:10.1093/mutage/gem012 PMID:17426049

Cavuşoğlu K, Yapar K, Oruç E, Yalçın E (2011). Protective effect of *Ginkgo biloba* L. leaf extract against glyphosate toxicity in Swiss albino mice. *J Med Food*, 14(10):1263–72. doi:10.1089/jmf.2010.0202 PMID:21859351

CCM International (2011). Outlook for China glyphosate industry 2012–2016. Available from: http://www.researchandmarkets.com/reports/2101356/outlook_for_china_glyphosate_industry_20122016, accessed 28 July 2015.

Centre de Toxicologie du Québec (1988). Etude de l'exposition professionelle des travailleurs forestiers exposés au glyphosate. Quebec: Le Centre Hospitalier de l'Université Laval. Available from: http://www.santecom.qc.ca/Bibliothequevirtuelle/santecom/35567000039898.pdf, accessed 28 July 2015. [French]

Chan P, Mahler J (1992). NTP technical report on the toxicity studies of glyphosate (CAS No. 1071-83-6) administered in dosed feed to F344/N rats and B6C3F1 mice. *Toxic Rep Ser*, 16:1–58. PMID:12209170

Chandra M, Frith CH (1994). Spontaneous renal lesions in CD-1 and B6C3F1 mice. *Exp Toxicol Pathol*, 46(3):189–98. doi:10.1016/S0940-2993(11)80080-1 PMID:8000238

Chang FC, Simcik MF, Capel PD (2011). Occurrence and fate of the herbicide glyphosate and its degradate aminomethylphosphonic acid in the atmosphere. *Environ Toxicol Chem*, 30(3):548–55. doi:10.1002/etc.431 PMID:21128261

Chaufan G, Coalova I, Ríos de Molina MC (2014). Glyphosate commercial formulation causes cytotoxicity, oxidative effects, and apoptosis on human cells: differences with its active ingredient. *Int J Toxicol*, 33(1):29–38. doi:10.1177/1091581813517906 PMID:24434723

Chen L, Xie M, Bi Y, Wang G, Deng S, Liu Y (2012). The combined effects of UV-B radiation and herbicides on photosynthesis, antioxidant enzymes and DNA damage in two bloom-forming cyanobacteria. *Ecotoxicol Environ Saf*, 80:224–30. doi:10.1016/j.ecoenv.2012.03.007 PMID:22464588

Chen M-X, Cao Z-Y, Jiang Y, Zhu Z-W (2013). Direct determination of glyphosate and its major metabolite, aminomethylphosphonic acid, in fruits and vegetables by mixed-mode hydrophilic interaction/weak anion-exchange liquid chromatography coupled with electrospray tandem mass spectrometry. *J Chromatogr A*, 1272:90–9. doi:10.1016/j.chroma.2012.11.069 PMID:23261284

Chen YJ, Wu ML, Deng JF, Yang CC (2009). The epidemiology of glyphosate-surfactant herbicide poisoning in Taiwan, 1986–2007: a poison center study. *Clin Toxicol (Phila)*, 47(7):670–7. doi:10.1080/15563650903140399 PMID:19640238

Chruscielska K, Brzezinski J, Kita K, Kalhorn D, Kita I, Graffstein B *et al.* (2000). Glyphosate - Evaluation of chronic activity and possible far-reaching effects. Part 1. Studies on chronic toxicity. *Pestycydy (Warsaw)*, 3–4:11–20.

Clair E, Mesnage R, Travert C, Séralini GÉ (2012). A glyphosate-based herbicide induces necrosis and apoptosis in mature rat testicular cells in vitro, and testosterone decrease at lower levels. *Toxicol In Vitro*, 26(2):269–79. doi:10.1016/j.tiv.2011.12.009 PMID:22200534

Clements C, Ralph S, Petras M (1997). Genotoxicity of select herbicides in *Rana catesbeiana* tadpoles using the alkaline single-cell gel DNA electrophoresis (comet) assay. *Environ Mol Mutagen*, 29(3):277–88. doi:10.1002/(SICI)1098-2280(1997)29:3<277::AID-EM8>3.0.CO;2-9 PMID:9142171

Coalova I, Ríos de Molina MC, Chaufan G (2014). Influence of the spray adjuvant on the toxicity effects of a glyphosate formulation. *Toxicol In Vitro*, 28(7):1306–11. doi:10.1016/j.tiv.2014.06.014 PMID:24999230

Cocco P, Satta G, Dubois S, Pili C, Pilleri M, Zucca M *et al.* (2013). Lymphoma risk and occupational exposure to pesticides: results of the Epilymph study. *Occup Environ Med*, 70(2):91–8. doi:10.1136/oemed-2012-100845 PMID:23117219

ColomboPage News Desk (2014). Sri Lanka lifts ban on sale of glyphosate. ColomboPage, Sri Lanka Internet Newspaper [online newspaper]. 13 May, 12:13 am Sri Lanka time. Available from: http://www.colombopage.com/archive_14A/May13_1399920230CH.php, accessed June 2015.

Conners DE, Black MC (2004). Evaluation of lethality and genotoxicity in the freshwater mussel *Utterbackia imbecillis* (Bivalvia: Unionidae) exposed singly and in combination to chemicals used in lawn care. *Arch Environ Contam Toxicol*, 46(3):362–71. doi:10.1007/s00244-003-3003-z PMID:15195808

Costa MJ, Monteiro DA, Oliveira-Neto AL, Rantin FT, Kalinin AL (2008). Oxidative stress biomarkers and heart function in bullfrog tadpoles exposed to Roundup Original. *Ecotoxicology*, 17(3):153–63. doi:10.1007/s10646-007-0178-5 PMID:17987383

Culbreth ME, Harrill JA, Freudenrich TM, Mundy WR, Shafer TJ (2012). Comparison of chemical-induced changes in proliferation and apoptosis in human and mouse neuroprogenitor cells. *Neurotoxicology*, 33(6):1499–510. doi:10.1016/j.neuro.2012.05.012 PMID:22634143

Curwin BD, Hein MJ, Sanderson WT, Nishioka MG, Reynolds SJ, Ward EM *et al.* (2005). Pesticide contamination inside farm and nonfarm homes. *J Occup Environ Hyg*, 2(7):357–67. doi:10.1080/15459620591001606 PMID:16020099

Curwin BD, Hein MJ, Sanderson WT, Striley C, Heederik D, Kromhout H *et al.* (2007). Urinary pesticide concentrations among children, mothers and fathers living in farm and non-farm households in Iowa. *Ann Occup Hyg*, 51(1):53–65. doi:10.1093/annhyg/mel062 PMID:16984946

de Castilhos Ghisi N, Cestari MM (2013). Genotoxic effects of the herbicide Roundup(®) in the fish *Corydoras paleatus* (Jenyns 1842) after short-term, environmentally low concentration exposure. *Environ Monit Assess*, 185(4):3201–7. doi:10.1007/s10661-012-2783-x PMID:22821326

De Marco A, De Simone C, Raglione M, Testa A, Trinca S (1992). Importance of the type of soil for the induction of micronuclei and the growth of primary roots of *Vicia faba* treated with the herbicides atrazine, glyphosate and maleic hydrazide. *Mutat Res*, 279(1):9–13. doi:10.1016/0165-1218(92)90260-7 PMID:1374535

de Menezes CC, da Fonseca MB, Loro VL, Santi A, Cattaneo R, Clasen B *et al.* (2011). Roundup effects on oxidative stress parameters and recovery pattern of *Rhamdia quelen*. *Arch Environ Contam Toxicol*, 60(4):665–71. doi:10.1007/s00244-010-9574-6 PMID:20680259

De Roos AJ, Blair A, Rusiecki JA, Hoppin JA, Svec M, Dosemeci M *et al.* (2005a). Cancer incidence among glyphosate-exposed pesticide applicators in the Agricultural Health Study. *Environ Health Perspect*, 113(1):49–54. doi:10.1289/ehp.7340 PMID:15626647

De Roos AJ, Svec MA, Blair A, Rusiecki JA, Dosemeci M, Alavanja MC *et al.* (2005b). Glyphosate results revisited: De Roos et al. respond. *Environ Health Perspect*, 113(6):A366–7. doi:10.1289/ehp.113-a366

De Roos AJ, Zahm SH, Cantor KP, Weisenburger DD, Holmes FF, Burmeister LF *et al.* (2003). Integrative assessment of multiple pesticides as risk factors for non-Hodgkin's lymphoma among men. *Occup Environ Med*, 60(9):E11 doi:10.1136/oem.60.9.e11 PMID:12937207

De Souza Filho J, Sousa CC, Da Silva CC, De Sabóia-Morais SM, Grisolia CK (2013). Mutagenicity and genotoxicity in gill erythrocyte cells of *Poecilia reticulata* exposed to a glyphosate formulation. *Bull Environ Contam Toxicol*, 91(5):583–7. doi:10.1007/s00128-013-1103-7 PMID:24042842

Dennis LK, Lynch CF, Sandler DP, Alavanja MC (2010). Pesticide use and cutaneous melanoma in pesticide applicators in the Agricultural Health Study. *Environ Health Perspect*, 118(6):812–7. doi:10.1289/ehp.0901518 PMID:20164001

Dill GM, Sammons RD, Feng PCC, Kohn F, Kretzmer K, Mehrsheikh A *et al.* (2010). Chapter 1: Glyphosate: discovery, development, applications, and properties. In: Nandula VK editor. *Glyphosate resistance in crops and weeds: history, development, and management.* Hoboken (NJ): Wiley; pp. 1–33.

Dimitrov BD, Gadeva PG, Benova DK, Bineva MV (2006). Comparative genotoxicity of the herbicides Roundup, Stomp and Reglone in plant and mammalian test systems. *Mutagenesis*, 21(6):375–82. doi:10.1093/mutage/gel044 PMID:16998229

dos Santos KC, Martinez CB (2014). Genotoxic and biochemical effects of atrazine and Roundup(®), alone and in combination, on the Asian clam *Corbicula fluminea*. *Ecotoxicol Environ Saf*, 100:7–14. doi:10.1016/j.ecoenv.2013.11.014 PMID:24433785

Duke SO, Powles SB (2009). Glyphosate-resistant crops and weeds. Now and in the future. *AgBioForum*, 12(3&4):346–57.

EFSA (2009). 2007 Annual Report on Pesticide Residues according to Article 32 of Regulation (EC) No 396/2005. Parma: European Food Safety Authority. Available from: http://www.efsa.europa.eu/en/efsajournal/pub/305r.htm, accessed 1 November 2014.

el-Gendy KS, Aly NM, el-Sebae AH (1998). Effects of edifenphos and glyphosate on the immune response and protein biosynthesis of bolti fish (*Tilapia nilotica*). *J Environ Sci Health B*, 33(2):135–49. doi:10.1080/03601239809373135 PMID:9536512

Elie-Caille C, Heu C, Guyon C, Nicod L (2010). Morphological damages of a glyphosate-treated human keratinocyte cell line revealed by a micro- to nanoscale microscopic investigation. *Cell Biol Toxicol*, 26(4):331–9. doi:10.1007/s10565-009-9146-6 PMID:20043237

Engel LS, Hill DA, Hoppin JA, Lubin JH, Lynch CF, Pierce J *et al.* (2005). Pesticide use and breast cancer risk among farmers' wives in the Agricultural Health Study. *Am J Epidemiol*, 161(2):121–35. doi:10.1093/aje/kwi022 PMID:15632262

EPA (1980a). Glyphosate; Submission of rat teratology, rabbit teratology, dominant lethal mutagenicity assay in mice. Washington (DC): United States Environmental Protection Agency, Office of Toxic substances. Available from: http://www.epa.gov/pesticides/chemicalsearch/chemical/foia/cleared-reviews/reviews/103601/103601-090.pdf, accessed 10 March 2015.

EPA (1980b). Review of Rodwell DE, Tasker EJ, Blair AM, *et al.* (1980). Teratology study in rats: IRDC No. 401–054. MRID 00046362. Washington (DC): United States Environmental Protection Agency. Available from: http://www.epa.gov/ncct/toxrefdb/, and from http://www.epa.gov/pesticides/chemicalsearch/chemical/foia/cleared-reviews/reviews/103601/103601-090.pdf, accessed 10 March 2015.

EPA (1983). Review of Knezevich A, Hogan G (1983). A chronic feeding study of glyphosate (Roundup Technical) in mice: Project No. 77–2061: Bdn-77- 420. Final Report. MRID 00130406. Washington (DC): United States Environmental Protection Agency. Available from: http://www.epa.gov/ncct/toxrefdb/, accessed 10 March 2015.

EPA (1985a). Glyphosate; EPA Reg.#: 524–308; Mouse oncogenicity study. Document No. 004370. Washington (DC): Office of Pesticides and Toxic Substances, United States Environmental Protection Agency. Available from: http://www.epa.gov/pesticides/chemicalsearch/chemical/foia/cleared-reviews/reviews/103601/103601-183.pdf, accessed 10 March 2015.

EPA (1985b). EPA Reg.#: 524–308; Roundup; glyphosate; pathology report on additional kidney sections. Document No. 004855. Washington (DC): Office of Pesticides and Toxic Substances, United States Environmental Protection Agency. Available from: http://www.epa.gov/pesticides/chemicalsearch/chemical/foia/cleared-reviews/reviews/103601/103601-206.pdf, accessed 10 March 2015.

EPA (1986). Glyphosate; EPA Registration No. 524–308; Roundup; additional histopathological evaluations of kidneys in the chronic feeding study of glyphosate in mice. Document No. 005590. Washington (DC): Office of Pesticides and Toxic Substances, United States Environmental Protection Agency. Available from: http://www.epa.gov/pesticides/chemicalsearch/chemical/foia/cleared-reviews/

reviews/103601/103601-211.pdf, accessed 10 March 2015.

EPA (1987). Review of Stout L, Johnson C (1987). 90-Day study of glyphosate administered in feed to Sprague-Dawley rats: Proj. ID ML-86–351/EHL 86128. MRID 40559401. Washington (DC): United States Environmental Protection Agency. Available from: http://www.epa.gov/ncct/toxrefdb/, accessed 10 March 2015.

EPA (1990). Review of Stout L, Ruecker F (1990). Chronic study of glyphosate administered in feed to albino rats: Laboratory Project Number: Msl-10495: RD 1014. MRID 41643801. Washington (DC): United States Environmental Protection Agency. Available from: http://www.epa.gov/ncct/toxrefdb/, accessed 10 March 2015.

EPA (1991a). Second peer review of glyphosate. Washington (DC): Office of Pesticides and Toxic Substances, United States Environmental Protection Agency. Available from: http://www.epa.gov/pesticides/chemicalsearch/chemical/foia/cleared-reviews/reviews/103601/103601-265.pdf, accessed 10 March 2015.

EPA (1991b). Glyphosate; 2-year combined chronic toxicity/carcinogenicity study in Sprague-Dawley rats - List A pesticide for reregistration. Document No. 008390. Washington (DC): Office of Pesticides and Toxic Substances, United States Environmental Protection Agency. Available from: http://www.epa.gov/pesticides/chemicalsearch/chemical/foia/cleared-reviews/reviews/103601/103601-263.pdf, accessed June 2015; see also http://www.epa.gov/pesticides/chemicalsearch/chemical/foia/cleared-reviews/reviews/103601/103601-268.pdf, accessed June 2015.

EPA (1991c). Peer review on glyphosate. Document No. 008527. Washington (DC): Office of Pesticides and Toxic Substances, United States Environmental Protection Agency.

EPA (1991d). Glyphosate – EPA registration No. 524–308 – 2-year chronic feeding/oncogenicity study in rats with technical glyphosate. Document No. 008897. Washington (DC): Office of Pesticides and Toxic Substances, United States Environmental Protection Agency. Available from: http://www.epa.gov/pesticides/chemicalsearch/chemical/foia/cleared-reviews/reviews/103601/103601-268.pdf, accessed 10 March 2015.

EPA (1992). Determination of glyphosate in drinking water by direct-aqueous-injection HPLC, post column derivatization, and fluorescence detection. In: Methods for the determination of organic compounds in drinking water - Supplement II (EPA/600/R-92–129). Washington (DC): Environmental Monitoring Systems Laboratory, Office of Research and Development, United States Environmental Protection Agency. Available through NTIS (http://www.ntis.gov).

EPA (1993a). Reregistration Eligibility Decision (RED): Glyphosate. EPA 738-R-93–014. Washington (DC): Office of Prevention, Pesticides And Toxic Substances, Office of Pesticide Programs, United States Environmental Protection Agency. Available from: http://www.epa.gov/opp00001/chem_search/reg_actions/reregistration/red_PC-417300_1-Sep-93.pdf, accessed 10 March 2015.

EPA (1993b). RED facts: Glyphosate. EPA-738-F-93–011. Washington (DC): Office of Prevention, Pesticides, and Toxic Substances, United States Environmental Protection Agency. Available from: http://www.epa.gov/oppsrrd1/reregistration/REDs/factsheets/0178fact.pdf, accessed 4 May 2015.

EPA (1997). Pesticides industry sales and usage –1994 and 1995 market estimates. Washington (DC): Biological and Economic Analysis Division, Office of Pesticide Programs, Office of Prevention, Pesticides And Toxic Substances, United States Environmental Protection Agency. Available from: http://www.epa.gov/pesticides/pestsales/95pestsales/market_estimates1995.pdf, accessed 10 March 2015.

EPA (2011). Pesticides industry sales and usage – 2006 and 2007 market estimates. Washington (DC): Biological and Economic Analysis Division, Office of Pesticide Programs, Office of Prevention, Pesticides And Toxic Substances, United States Environmental Protection Agency. Available from: http://www.epa.gov/opp00001/pestsales/07pestsales/market_estimates2007.pdf, accessed 10 March 2015.

EPA (2015). Toxicity Reference Database (ToxRefDB). Computational Toxicology Research Program, United States Environmental Protection Agency. Available from: http://www.epa.gov/ncct/toxrefdb/, accessed 10 March 2015.

Eriksson M, Hardell L, Carlberg M, Akerman M (2008). Pesticide exposure as risk factor for non-Hodgkin lymphoma including histopathological subgroup analysis. *Int J Cancer*, 123(7):1657–63. doi:10.1002/ijc.23589 PMID:18623080

European Commission (2002). Review report for the active substance glyphosate (6511/VI/99-final, 21 January 2002). Brussels: Health and Consumer Protection Directorate-General, European Commission. Available from: http://ec.europa.eu/food/plant/protection/evaluation/existactive/list1_glyphosate_en.pdf, accessed 29 April 2015.

Eustis SL, Hailey JR, Boorman GA, Haseman JK (1994). The utility of multiple-section sampling in the histopathological evaluation of the kidney for carcinogenicity studies. *Toxicol Pathol*, 22(5):457–72. doi:10.1177/019262339402200501 PMID:7899775

FAO (2000). Glyphosate, N-(phosphonomethyl)glycine. Specifications and evaluations for plant protection products. Rome: Food and Agriculture Organization of the United Nations. Available from: http://www.fao.

org/fileadmin/templates/agphome/documents/Pests_Pesticides/Specs/glypho01.pdf, accessed 28 July 2015.

Farm Chemicals International (2015). Glyphosate. In: Crop Protection Database. Willoughby (OH): Meister Media Worldwide. Available from: http://www.farmchemicalsinternational.com/crop-protection-database/#/product/detail/203900/, accessed 2 February 2015.

Ferreira D, da Motta AC, Kreutz LC, Toni C, Loro VL, Barcellos LJ (2010). Assessment of oxidative stress in *Rhamdia quelen* exposed to agrichemicals. *Chemosphere*, 79(9):914–21. doi:10.1016/j.chemosphere.2010.03.024 PMID:20371099

Flower KB, Hoppin JA, Lynch CF, Blair A, Knott C, Shore DL *et al.* (2004). Cancer risk and parental pesticide application in children of Agricultural Health Study participants. *Environ Health Perspect*, 112(5):631–5. doi:10.1289/ehp.6586 PMID:15064173

Forgacs AL, Ding Q, Jaremba RG, Huhtaniemi IT, Rahman NA, Zacharewski TR (2012). BLTK1 murine Leydig cells: a novel steroidogenic model for evaluating the effects of reproductive and developmental toxicants. *Toxicol Sci*, 127(2):391–402. doi:10.1093/toxsci/kfs121 PMID:22461451

Freedonia (2012). World agricultural pesticides: industry study with forecasts for 2016 & 2021. Study #2902, August 2012. Cleveland (OH): The Freedonia Group. Available from: http://www.freedoniagroup.com/brochure/29xx/2902smwe.pdf, accessed 10 March 2015.

Frescura VD, Kuhn AW, Laughinghouse HD 4th, Paranhos JT, Tedesco SB (2013). Post-treatment with plant extracts used in Brazilian folk medicine caused a partial reversal of the antiproliferative effect of glyphosate in the *Allium cepa* test. *Biocell*, 37(2):23–8. PMID:24392578

Gasnier C, Benachour N, Clair E, Travert C, Langlois F, Laurant C *et al.* (2010). Dig1 protects against cell death provoked by glyphosate-based herbicides in human liver cell lines. *J Occup Med Toxicol*, 5(1):29 doi:10.1186/1745-6673-5-29 PMID:20979644

Gasnier C, Dumont C, Benachour N, Clair E, Chagnon MC, Séralini GE (2009). Glyphosate-based herbicides are toxic and endocrine disruptors in human cell lines. *Toxicology*, 262(3):184–91. doi:10.1016/j.tox.2009.06.006 PMID:19539684

Gehin A, Guillaume YC, Millet J, Guyon C, Nicod L (2005). Vitamins C and E reverse effect of herbicide-induced toxicity on human epidermal cells HaCaT: a biochemometric approach. *Int J Pharm*, 288(2):219–26. doi:10.1016/j.ijpharm.2004.09.024 PMID:15620861

George J, Prasad S, Mahmood Z, Shukla Y (2010). Studies on glyphosate-induced carcinogenicity in mouse skin: a proteomic approach. *J Proteomics*, 73(5):951–64. doi:10.1016/j.jprot.2009.12.008 PMID:20045496

George J, Shukla Y (2013). Emptying of intracellular calcium pool and oxidative stress imbalance are associated with the glyphosate-induced proliferation in human skin keratinocytes HaCaT cells. *ISRN Dermatol*, 2013:825180 doi:10.1155/2013/825180 PMID:24073338

Geret F, Burgeot T, Haure J, Gagnaire B, Renault T, Communal PY *et al.* (2013). Effects of low-dose exposure to pesticide mixture on physiological responses of the Pacific oyster, Crassostrea gigas. *Environ Toxicol*, 28(12):689–99. doi:10.1002/tox.20764 PMID:22012874

Gholami-Seyedkolaei SJ, Mirvaghefi A, Farahmand H, Kosari AA, Gholami-Seyedkolaei SJ, Gholami-Seyedkolaei SJ (2013). Optimization of recovery patterns in common carp exposed to Roundup using response surface methodology: evaluation of neurotoxicity and genotoxicity effects and biochemical parameters. *Ecotoxicol Environ Saf*, 98:152–61. doi:10.1016/j.ecoenv.2013.09.009 PMID:24094415

Glusczak L, Loro VL, Pretto A, Moraes BS, Raabe A, Duarte MF *et al.* (2011). Acute exposure to glyphosate herbicide affects oxidative parameters in piava (*Leporinus obtusidens*). *Arch Environ Contam Toxicol*, 61(4):624–30. doi:10.1007/s00244-011-9652-4 PMID:21465245

Glyphosate Task Force (2014). How is glyphosate used? Glyphosate facts. Updated 10 March 2014. Darmstadt: Industry Task Force on Glyphosate. Available from: http://www.glyphosate.eu/how-glyphosate-used, accessed 21 April 2015.

Granby K, Vahl M (2001). Investigation of the herbicide glyphosate and the plant growth regulators chlormequat and mepiquat in cereals produced in Denmark. *Food Addit Contam*, 18(10):898–905. doi:10.1080/02652030119594 PMID:11569770

Greim H, Saltmiras D, Mostert V, Strupp C (2015). Evaluation of carcinogenic potential of the herbicide glyphosate, drawing on tumor incidence data from fourteen chronic/carcinogenicity rodent studies. *Crit Rev Toxicol*, 45(3):185–208. doi:10.3109/10408444.2014.1003423 PMID:25716480

Grisolia CK (2002). A comparison between mouse and fish micronucleus test using cyclophosphamide, mitomycin C and various pesticides. *Mutat Res*, 518(2):145–50. doi:10.1016/S1383-5718(02)00086-4 PMID:12113765

Guha N, Ward MH, Gunier R, Colt JS, Lea CS, Buffler PA *et al.* (2013). Characterization of residential pesticide use and chemical formulations through self-report and household inventory: the Northern California Childhood Leukemia study. *Environ Health Perspect*, 121(2):276–82. PMID:23110983

Gui YX, Fan XN, Wang HM, Wang G, Chen SD (2012). Glyphosate induced cell death through apoptotic and autophagic mechanisms. *Neurotoxicol Teratol*, 34(3):344–9. doi:10.1016/j.ntt.2012.03.005 PMID:22504123

Guilherme S, Gaivão I, Santos MA, Pacheco M (2010). European eel (*Anguilla anguilla*) genotoxic and pro-oxidant responses following short-term exposure to Roundup–a glyphosate-based herbicide. *Mutagenesis*, 25(5):523–30. doi:10.1093/mutage/geq038 PMID:20643706

Guilherme S, Gaivão I, Santos MA, Pacheco M (2012a). DNA damage in fish (*Anguilla anguilla*) exposed to a glyphosate-based herbicide – elucidation of organ-specificity and the role of oxidative stress. *Mutat Res*, 743(1–2):1–9. doi:10.1016/j.mrgentox.2011.10.017 PMID:22266476

Guilherme S, Santos MA, Barroso C, Gaivão I, Pacheco M (2012b). Differential genotoxicity of Roundup(®) formulation and its constituents in blood cells of fish (*Anguilla anguilla*): considerations on chemical interactions and DNA damaging mechanisms. *Ecotoxicology*, 21(5):1381–90. doi:10.1007/s10646-012-0892-5 PMID:22526921

Guilherme S, Santos MA, Gaivão I, Pacheco M (2014a). Are DNA-damaging effects induced by herbicide formulations (Roundup® and Garlon®) in fish transient and reversible upon cessation of exposure? *Aquat Toxicol*, 155:213–21. doi:10.1016/j.aquatox.2014.06.007 PMID:25058560

Guilherme S, Santos MA, Gaivão I, Pacheco M (2014b). DNA and chromosomal damage induced in fish (*Anguilla anguilla* L.) by aminomethylphosphonic acid (AMPA)–the major environmental breakdown product of glyphosate. *Environ Sci Pollut Res Int*, 21(14):8730–9. doi:10.1007/s11356-014-2803-1 PMID:24696215

Hardell L, Eriksson M (1999). A case-control study of non-Hodgkin lymphoma and exposure to pesticides. *Cancer*, 85(6):1353–60. doi:10.1002/(SICI)1097-0142(19990315)85:6<1353::AID-CNCR19>3.0.CO;2-1 PMID:10189142

Hardell L, Eriksson M, Nordstrom M (2002). Exposure to pesticides as risk factor for non-Hodgkin's lymphoma and hairy cell leukemia: pooled analysis of two Swedish case-control studies. *Leuk Lymphoma*, 43(5):1043–9. PMID:12148884

Hayes WJ Jr, Laws ER Jr editors. (1991). *Classes of pesticides*. Handbook of Pesticide Toxicology. Volume 3:. New York (NY): Academic Press, Inc.; p. 1340.

Heu C, Elie-Caille C, Mougey V, Launay S, Nicod L (2012). A step further toward glyphosate-induced epidermal cell death: involvement of mitochondrial and oxidative mechanisms. *Environ Toxicol Pharmacol*, 34(2):144–53. doi:10.1016/j.etap.2012.02.010 PMID:22522424

Hidalgo C, Rios C, Hidalgo M, Salvadó V, Sancho JV, Hernández F (2004). Improved coupled-column liquid chromatographic method for the determination of glyphosate and aminomethylphosphonic acid residues in environmental waters. *J Chromatogr A*, 1035(1):153–7. doi:10.1016/j.chroma.2004.02.044 PMID:15117086

Hilton CW (2012). Monsanto & the global glyphosate market: case study. The Wiglaf Journal. June 2012. Available from: http://www.wiglafjournal.com/pricing/2012/06/monsanto-the-global-glyphosate-market-case-study/, accessed 28 July 2015.

Hoar SK, Blair A, Holmes FF, Boysen CD, Robel RJ, Hoover R *et al.* (1986). Agricultural herbicide use and risk of lymphoma and soft-tissue sarcoma. *JAMA*, 256(9):1141–7. doi:10.1001/jama.1986.03380090081023 PMID:3801091

Humphries D, Byrtus G, Anderson AM (2005). Glyphosate residues in Alberta's atmospheric deposition, soils and surface waters. Alberta: Water Research Users Group, Alberta Environment. Available from: http://environment.gov.ab.ca/info/library/6444.pdf, accessed 13 November 2014.

IARC (2006). Data for the Monographs. In: Preamble to the IARC Monographs (amended January 2006). Lyon: International Agency for Research on Cancer. Available from: http://monographs.iarc.fr/ENG/Preamble/index.php, accessed 28 July 2015.

IARC (2014). Table 1. Key characteristics of carcinogens. In: Instructions for authors. Lyon: International Agency for Research on Cancer. Available from: http://monographs.iarc.fr/ENG/Preamble/previous/Instructions_to_Authors_S4.pdf, accessed 28 July 2015.

IPCS (1994). Glyphosate. Environmental Health Criteria 159. Geneva: International Programme on Chemical Safety, World Health Organization. Available from: http://www.inchem.org/documents/ehc/ehc/ehc159.htm, accessed 28 July 2015.

IPCS (1996). Glyphosate. WHO/FAO Data Sheets on Pesticides, No. 91 (WHO/PCS/DS/96.91). Geneva: International Programme on Chemical Safety, World Health Organization. Available from: http://apps.who.int/iris/handle/10665/63290.

IPCS (2005). Glyphosate. International Chemical Safety Card (ICSC 0160). Geneva: International Programme on Chemical Safety, World Health Organization. Available from: http://www.inchem.org/documents/icsc/icsc/eics0160.htm, accessed 2 February 2015.

Jacob GS, Garbow JR, Hallas LE, Kimack NM, Kishore GM, Schaefer J (1988). Metabolism of glyphosate in Pseudomonas sp. strain LBr. *Appl Environ Microbiol*, 54(12):2953–8. PMID:3223761

Jan MR, Shah J, Muhammad M, Ara B (2009). Glyphosate herbicide residue determination in samples of environmental importance using spectrophotometric method. *J Hazard Mater*, 169(1–3):742–5. doi:10.1016/j.jhazmat.2009.04.003 PMID:19411135

Jasper R, Locatelli GO, Pilati C, Locatelli C (2012). Evaluation of biochemical, hematological and oxidative parameters in mice exposed to the herbicide glyphosate-Roundup(®). *Interdiscip Toxicol*, 5(3):133–40. doi:10.2478/v10102-012-0022-5 PMID:23554553

Jauhiainen A, Räsänen K, Sarantila R, Nuutinen J, Kangas J (1991). Occupational exposure of forest workers to glyphosate during brush saw spraying work. *Am Ind Hyg*

Assoc J, 52(2):61–4. doi:10.1080/15298669191364334 PMID:2011980

JMPR (2006). Glyphosate. In: Joint FAO/WHO Meeting on Pesticide Residues. Pesticide residues in food – 2004: toxicological evaluations. Report No. WHO/PCS/06.1. Geneva: World Health Organization; pp. 95–169. Available from: http://whqlibdoc.who.int/publications/2006/9241665203_eng.pdf?ua=1, accessed 6 March 2015.

Johnson PD, Rimmer DA, Garrod AN, Helps JE, Mawdsley C (2005). Operator exposure when applying amenity herbicides by all-terrain vehicles and controlled droplet applicators. *Ann Occup Hyg*, 49(1):25–32. PMID:15596423

Kachuri L, Demers PA, Blair A, Spinelli JJ, Pahwa M, McLaughlin JR *et al.* (2013). Multiple pesticide exposures and the risk of multiple myeloma in Canadian men. *Int J Cancer*, 133(8):1846–58. doi:10.1002/ijc.28191 PMID:23564249

Kale PG, Petty BT Jr, Walker S, Ford JB, Dehkordi N, Tarasia S *et al.* (1995). Mutagenicity testing of nine herbicides and pesticides currently used in agriculture. *Environ Mol Mutagen*, 25(2):148–53. doi:10.1002/em.2850250208 PMID:7698107

Kalyanaraman B, Darley-Usmar V, Davies KJ, Dennery PA, Forman HJ, Grisham MB *et al.* (2012). Measuring reactive oxygen and nitrogen species with fluorescent probes: challenges and limitations. *Free Radic Biol Med*, 52(1):1–6. doi:10.1016/j.freeradbiomed.2011.09.030 PMID:22027063

Karunanayake CP, Spinelli JJ, McLaughlin JR, Dosman JA, Pahwa P, McDuffie HH (2012). Hodgkin lymphoma and pesticides exposure in men: a Canadian case-control study. *J Agromed*, 17(1):30–9. doi:10.1080/1059924X.2012.632726 PMID:22191501

Kavlock R, Chandler K, Houck K, Hunter S, Judson R, Kleinstreuer N *et al.* (2012). Update on EPA's ToxCast program: providing high throughput decision support tools for chemical risk management. *Chem Res Toxicol*, 25(7):1287–302. doi:10.1021/tx3000939 PMID:22519603

Kaya B, Creus A, Yanikoğlu A, Cabré O, Marcos R (2000). Use of the Drosophila wing spot test in the genotoxicity testing of different herbicides. *Environ Mol Mutagen*, 36(1):40–6. doi:10.1002/1098-2280(2000)36:1<40::AID-EM6>3.0.CO;2-K PMID:10918358

Kier LD, Kirkland DJ (2013). Review of genotoxicity studies of glyphosate and glyphosate-based formulations. *Crit Rev Toxicol*, 43(4):283–315. doi:10.3109/10408444.2013.770820 PMID:23480780

Kim YH, Hong JR, Gil HW, Song HY, Hong SY (2013). Mixtures of glyphosate and surfactant TN20 accelerate cell death via mitochondrial damage-induced apoptosis and necrosis. *Toxicol In Vitro*, 27(1):191–7. doi:10.1016/j.tiv.2012.09.021 PMID:23099315

Kojima H, Katsura E, Takeuchi S, Niiyama K, Kobayashi K (2004). Screening for estrogen and androgen receptor activities in 200 pesticides by in vitro reporter gene assays using Chinese hamster ovary cells. *Environ Health Perspect*, 112(5):524–31. doi:10.1289/ehp.6649 PMID:15064155

Kojima H, Takeuchi S, Nagai T (2010). Endocrine-disrupting potential of pesticides via nuclear receptors and aryl hydrocarbon receptor *J Health Sci*, 56(4):374–86. doi:10.1248/jhs.56.374

Koller VJ, Fürhacker M, Nersesyan A, Mišík M, Eisenbauer M, Knasmueller S (2012). Cytotoxic and DNA-damaging properties of glyphosate and Roundup in human-derived buccal epithelial cells. *Arch Toxicol*, 86(5):805–13. doi:10.1007/s00204-012-0804-8 PMID:22331240

Kolpin DW, Thurman EM, Lee EA, Meyer MT, Furlong ET, Glassmeyer ST (2006). Urban contributions of glyphosate and its degradate AMPA to streams in the United States. *Sci Total Environ*, 354(2–3):191–7. doi:10.1016/j.scitotenv.2005.01.028 PMID:16398995

Kreutz LC, Gil Barcellos LJ, de Faria Valle S, de Oliveira Silva T, Anziliero D, Davi dos Santos E *et al.* (2011). Altered hematological and immunological parameters in silver catfish (*Rhamdia quelen*) following short term exposure to sublethal concentration of glyphosate. *Fish Shellfish Immunol*, 30(1):51–7. doi:10.1016/j.fsi.2010.09.012 PMID:20883798

Kuang H, Wang L, Xu C (2011). Overview of analytical techniques for herbicides in foods. In: Soloneski S, Larramendy ML, editors. Herbicides, theory and applications. Available from: http://www.intechopen.com/books/herbicides-theory-and-applications, accessed 28 July 2015.

Kumar S, Khodoun M, Kettleson EM, McKnight C, Reponen T, Grinshpun SA *et al.* (2014). Glyphosate-rich air samples induce IL-33, TSLP and generate IL-13 dependent airway inflammation. *Toxicology*, 325:42–51. doi:10.1016/j.tox.2014.08.008 PMID:25172162

Kwiatkowska M, Huras B, Bukowska B (2014). The effect of metabolites and impurities of glyphosate on human erythrocytes (in vitro). *Pestic Biochem Physiol*, 109:34–43. doi:10.1016/j.pestbp.2014.01.003 PMID:24581382

Landgren O, Kyle RA, Hoppin JA, Beane Freeman LE, Cerhan JR, Katzmann JA *et al.* (2009). Pesticide exposure and risk of monoclonal gammopathy of undetermined significance in the Agricultural Health Study. *Blood*, 113(25):6386–91. doi:10.1182/blood-2009-02-203471 PMID:19387005

Larsen K, Najle R, Lifschitz A, Maté ML, Lanusse C, Virkel GL (2014). Effects of sublethal exposure to a glyphosate-based herbicide formulation on metabolic activities of different xenobiotic-metabolizing enzymes in rats. *Int J Toxicol*, 33(4):307–18. doi:10.1177/1091581814540481 PMID:24985121

Lavy TL, Cowell JE, Steinmetz JR, Massey JH (1992). Conifer seedling nursery worker exposure to

glyphosate. *Arch Environ Contam Toxicol*, 22(1):6–13. doi:10.1007/BF00213295 PMID:1554254
Lee EA, Strahan AP, Thurman EM (2001). Methods of analysis by the U.S. Geological Survey Organic Geochemistry Research Group — determination of glyphosate, aminomethylphophonic acid, and glufosinate in water using online solid-phase extraction and high-performance liquid chromatography/mass spectrometry. Open-File Report 01–454. Lawrence (KS): United States Geological Survey. Available from: http://ks.water.usgs.gov/pubs/reports/ofr.01-454.pdf, accessed 28 July 2015.
Lee WJ, Cantor KP, Berzofsky JA, Zahm SH, Blair A (2004a). Non-Hodgkin's lymphoma among asthmatics exposed to pesticides. *Int J Cancer*, 111(2):298–302. doi:10.1002/ijc.20273 PMID:15197786
Lee WJ, Colt JS, Heineman EF, McComb R, Weisenburger DD, Lijinsky W *et al.* (2005). Agricultural pesticide use and risk of glioma in Nebraska, United States. *Occup Environ Med*, 62(11):786–92. doi:10.1136/oem.2005.020230 PMID:16234405
Lee WJ, Lijinsky W, Heineman EF, Markin RS, Weisenburger DD, Ward MH (2004b). Agricultural pesticide use and adenocarcinomas of the stomach and oesophagus. *Occup Environ Med*, 61(9):743–9. doi:10.1136/oem.2003.011858 PMID:15317914
Lee WJ, Sandler DP, Blair A, Samanic C, Cross AJ, Alavanja MC (2007). Pesticide use and colorectal cancer risk in the Agricultural Health Study. *Int J Cancer*, 121(2):339–46. doi:10.1002/ijc.22635 PMID:17390374
Li AP, Long TJ (1988). An evaluation of the genotoxic potential of glyphosate. *Fundam Appl Toxicol*, 10(3):537–46. doi:10.1016/0272-0590(88)90300-4 PMID:3286348
Li Q, Lambrechts MJ, Zhang Q, Liu S, Ge D, Yin R *et al.* (2013). Glyphosate and AMPA inhibit cancer cell growth through inhibiting intracellular glycine synthesis. *Drug Des Dev Ther*, 7:635–43. PMID:23983455
Lioi MB, Scarfì MR, Santoro A, Barbieri R, Zeni O, Di Berardino D *et al.* (1998). Genotoxicity and oxidative stress induced by pesticide exposure in bovine lymphocyte cultures in vitro. *Mutat Res*, 403(1–2):13–20. doi:10.1016/S0027-5107(98)00010-4 PMID:9726001
Lopes FM, Varela Junior AS, Corcini CD, da Silva AC, Guazzelli VG, Tavares G *et al.* (2014). Effect of glyphosate on the sperm quality of zebrafish *Danio rerio*. *Aquat Toxicol*, 155:322–6. doi:10.1016/j.aquatox.2014.07.006 PMID:25089920
Lubick N (2009). Environmental impact of cocaine strategy assessed [News]*Nature*, Published online 12 November, doi doi:10.1038/news.2009.1080
Lueken A, Juhl-Strauss U, Krieger G, Witte I (2004). Synergistic DNA damage by oxidative stress (induced by H_2O_2) and nongenotoxic environmental chemicals in human fibroblasts. *Toxicol Lett*, 147(1):35–43. doi:10.1016/j.toxlet.2003.10.020 PMID:14700526
Lushchak OV, Kubrak OI, Storey JM, Storey KB, Lushchak VI (2009). Low toxic herbicide Roundup induces mild oxidative stress in goldfish tissues. *Chemosphere*, 76(7):932–7. doi:10.1016/j.chemosphere.2009.04.045 PMID:19450865
Mahendrakar K, Venkategowda PM, Rao SM, Mutkule DP (2014). Glyphosate surfactant herbicide poisoning and management. *Indian J Crit Care Med*, 18(5):328–30. doi:10.4103/0972-5229.132508 PMID:24914265
Malatesta M, Perdoni F, Santin G, Battistelli S, Muller S, Biggiogera M (2008). Hepatoma tissue culture (HTC) cells as a model for investigating the effects of low concentrations of herbicide on cell structure and function. *Toxicol In Vitro*, 22(8):1853–60. doi:10.1016/j.tiv.2008.09.006 PMID:18835430
Mañas F, Peralta L, Raviolo J, García Ovando H, Weyers A, Ugnia L *et al.* (2009b). Genotoxicity of AMPA, the environmental metabolite of glyphosate, assessed by the Comet assay and cytogenetic tests. *Ecotoxicol Environ Saf*, 72(3):834–7. doi:10.1016/j.ecoenv.2008.09.019 PMID:19013644
Mañas F, Peralta L, Raviolo J, Ovando HG, Weyers A, Ugnia L *et al.* (2009a). Genotoxicity of glyphosate assessed by the comet assay and cytogenetic tests. *Environ Toxicol Pharmacol*, 28(1):37–41. doi:10.1016/j.etap.2009.02.001 PMID:21783980
Mance D 3rd (2012). The great glyphosate debate. *Northern Woodlands* [online magazine]. 8 March. Available from: http://northernwoodlands.org/articles/article/the-great-glyphosate-debate, accessed 28 July 2015.
Mariager TP, Madsen PV, Ebbehøj NE, Schmidt B, Juhl A (2013). Severe adverse effects related to dermal exposure to a glyphosate-surfactant herbicide. *Clin Toxicol (Phila)*, 51(2):111–3. doi:10.3109/15563650.2013.763951 PMID:23360343
Marques A, Guilherme S, Gaivão I, Santos MA, Pacheco M (2014). Progression of DNA damage induced by a glyphosate-based herbicide in fish (*Anguilla anguilla*) upon exposure and post-exposure periods–insights into the mechanisms of genotoxicity and DNA repair. *Comp Biochem Physiol C Toxicol Pharmacol*, 166:126–33. doi:10.1016/j.cbpc.2014.07.009 PMID:25110831
Marques A, Guilherme S, Gaivão I, Santos MA, Pacheco M (2015). Erratum to: "Progression of DNA damage induced by a glyphosate-based herbicide in fish (*Anguilla anguilla*) upon exposure and post-exposure periods - Insights into the mechanisms of genotoxicity and DNA repair" [Comp. Biochem. Physiol. C 166 (2014) 126–133]. *Comp Biochem Physiol C Toxicol Pharmacol*, 168C:1 doi:10.1016/j.cbpc.2014.10.008 PMID:25521452
Martini CN, Gabrielli M, Vila MC (2012). A commercial formulation of glyphosate inhibits proliferation and differentiation to adipocytes and induces apoptosis in 3T3-L1 fibroblasts. *Toxicol In Vitro*, 26(6):1007–13. doi:10.1016/j.tiv.2012.04.017 PMID:22546541

McDuffie HH, Pahwa P, McLaughlin JR, Spinelli JJ, Fincham S, Dosman JA *et al.* (2001). Non-Hodgkin's lymphoma and specific pesticide exposures in men: cross-Canada study of pesticides and health. *Cancer Epidemiol Biomarkers Prev*, 10(11):1155–63. PMID:11700263

McQueen H, Callan AC, Hinwood AL (2012). Estimating maternal and prenatal exposure to glyphosate in the community setting. *Int J Hyg Environ Health*, 215(6):570–6. doi:10.1016/j.ijheh.2011.12.002 PMID:22261298

Mesnage R, Bernay B, Séralini GE (2013). Ethoxylated adjuvants of glyphosate-based herbicides are active principles of human cell toxicity. *Toxicology*, 313(2–3):122–8. doi:10.1016/j.tox.2012.09.006 PMID:23000283

Meza-Joya FL, Ramírez-Pinilla MP, Fuentes-Lorenzo JL (2013). Toxic, cytotoxic, and genotoxic effects of a glyphosate formulation (Roundup®SL-Cosmoflux®411F) in the direct-developing frog *Eleutherodactylus johnstonei*. *Environ Mol Mutagen*, 54(5):362–73. doi:10.1002/em.21775 PMID:23625742

Ministry of Chemicals & Fertilizers (2008). Performance of chemical & petrochemical industry at a glance (2001–2007). New Delhi: Monitoring and Evaluation Division, Department of Chemicals and Petrochemicals, Government of India. Available from: http://chemicals.nic.in/stat0107.pdf, accessed February 2015.

Mladinic M, Berend S, Vrdoljak AL, Kopjar N, Radic B, Zeljezic D (2009b). Evaluation of genome damage and its relation to oxidative stress induced by glyphosate in human lymphocytes in vitro. *Environ Mol Mutagen*, 50(9):800–7. doi:10.1002/em.20495 PMID:19402152

Mladinic M, Perkovic P, Zeljezic D (2009a). Characterization of chromatin instabilities induced by glyphosate, terbuthylazine and carbofuran using cytome FISH assay. *Toxicol Lett*, 189(2):130–7. doi:10.1016/j.toxlet.2009.05.012 PMID:19477249

MLHB (2013). Determination of glyphosate residues in human urine samples from 18 European countries. Bremen: Medical Laboratory of Bremen. Available from: https://www.foeeurope.org/sites/default/files/glyphosate_studyresults_june12.pdf, accessed 24 November 2014.

Modesto KA, Martinez CB (2010a). Effects of Roundup Transorb on fish: hematology, antioxidant defenses and acetylcholinesterase activity. *Chemosphere*, 81(6):781–7. doi:10.1016/j.chemosphere.2010.07.005 PMID:20684975

Modesto KA, Martinez CB (2010b). Roundup causes oxidative stress in liver and inhibits acetylcholinesterase in muscle and brain of the fish *Prochilodus lineatus*. *Chemosphere*, 78(3):294–9. doi:10.1016/j.chemosphere.2009.10.047 PMID:19910015

Mohamed AH (2011). Sublethal toxicity of Roundup to immunological and molecular aspects of *Biomphalaria alexandrina* to *Schistosoma mansoni* infection. *Ecotoxicol Environ Saf*, 74(4):754–60. doi:10.1016/j.ecoenv.2010.10.037 PMID:21126764

Monge P, Wesseling C, Guardado J, Lundberg I, Ahlbom A, Cantor KP *et al.* (2007). Parental occupational exposure to pesticides and the risk of childhood leukemia in Costa Rica. *Scand J Work Environ Health*, 33(4):293–303. doi:10.5271/sjweh.1146 PMID:17717622

Monroy CM, Cortés AC, Sicard DM, de Restrepo HG (2005). [Cytotoxicity and genotoxicity of human cells exposed in vitro to glyphosate] *Biomedica*, 25(3):335–45. doi:10.7705/biomedica.v25i3.1358 PMID:16276681

Moreno NC, Sofia SH, Martinez CB (2014). Genotoxic effects of the herbicide Roundup Transorb and its active ingredient glyphosate on the fish *Prochilodus lineatus*. *Environ Toxicol Pharmacol*, 37(1):448–54. doi:10.1016/j.etap.2013.12.012 PMID:24448465

Mortensen OS, Sørensen FW, Gregersen M, Jensen K (2000). [Poisonings with the herbicides glyphosate and glyphosate-trimesium] [in Danish] *Ugeskr Laeger*, 162(35):4656–9. PMID:10986892

Motojyuku M, Saito T, Akieda K, Otsuka H, Yamamoto I, Inokuchi S (2008). Determination of glyphosate, glyphosate metabolites, and glufosinate in human serum by gas chromatography-mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci*, 875(2):509–14. doi:10.1016/j.jchromb.2008.10.003 PMID:18945648

Muangphra P, Kwankua W, Gooneratne R (2014). Genotoxic effects of glyphosate or paraquat on earthworm coelomocytes. *Environ Toxicol*, 29(6):612–20. doi:10.1002/tox.21787 PMID:22644885

Nakashima K, Yoshimura T, Mori H, Kawaguchi M, Adachi S, Nakao T *et al.* (2002). [Effects of pesticides on cytokine production by human peripheral blood mononuclear cells–fenitrothion and glyphosate] *Chudoku Kenkyu*, 15(2):159–65. PMID:12108020

NCBI (2015). Glyphosate. Compound summary for CID 3496. PubChem Open Chemistry Database. Bethesda (MD): National Center for Biotechnology Information, United States National Library of Medicine. Available from: http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=3496, accessed 5 March 2015.

Nedelkoska TV, Low GKC (2004). High-performance liquid chromatographic determination of glyphosate in water and plant material after pre-column derivatisation with 9-fluorenylmethyl chloroformate. *Anal Chim Acta*, 511(1):145–53. doi:10.1016/j.aca.2004.01.027

NIH (2015). Questionnaires and study data. Agricultural Health Study. National Institutes of Health. Available from: http://aghealth.nih.gov/collaboration/questionnaires.html, accessed 12 June 2015.

Nordström M, Hardell L, Magnuson A, Hagberg H, Rask-Andersen A (1998). Occupational exposures, animal exposure and smoking as risk factors for hairy cell leukaemia evaluated in a case-control study. *Br*

J Cancer, 77(11):2048–52. doi:10.1038/bjc.1998.341 PMID:9667691

NPIC (2010). Glyphosate. General fact sheet. Oregon State University: National Pesticide Information Center. Available from: http://npic.orst.edu/factsheets/glyphogen.pdf, accessed June 2015.

Nwani CD, Nagpure NS, Kumar R, Kushwaha B, Lakra WS (2013). DNA damage and oxidative stress modulatory effects of glyphosate-based herbicide in freshwater fish, *Channa punctatus*. *Environ Toxicol Pharmacol*, 36(2):539–47. doi:10.1016/j.etap.2013.06.001 PMID:23816461

Omran NE, Salama WM (2013). The endocrine disrupter effect of atrazine and glyphosate on *Biomphalaria alexandrina* snails. *Toxicol Ind Health*, doi:10.1177/0748233713506959 PMID:24215068

Orsi L, Delabre L, Monnereau A, Delval P, Berthou C, Fenaux P *et al.* (2009). Occupational exposure to pesticides and lymphoid neoplasms among men: results of a French case-control study. *Occup Environ Med*, 66(5):291–8. doi:10.1136/oem.2008.040972 PMID:19017688

Ortiz-Ordoñez E, Uría-Galicia E, Ruiz-Picos RA, Duran AG, Trejo YH, Sedeño-Díaz JE *et al.* (2011). Effect of Yerbimat herbicide on lipid peroxidation, catalase activity, and histological damage in gills and liver of the freshwater fish *Goodea atripinnis*. *Arch Environ Contam Toxicol*, 61(3):443–52. doi:10.1007/s00244-011-9648-0 PMID:21305274

Paganelli A, Gnazzo V, Acosta H, López SL, Carrasco AE (2010). Glyphosate-based herbicides produce teratogenic effects on vertebrates by impairing retinoic acid signalling. *Chem Res Toxicol*, 23(10):1586–95. doi:10.1021/tx1001749 PMID:20695457

Pahwa P, Karunanayake CP, Dosman JA, Spinelli JJ, McLaughlin JR; Cross-Canada Group(2011). Soft-tissue sarcoma and pesticides exposure in men: results of a Canadian case-control study. *J Occup Environ Med*, 53(11):1279–86. doi:10.1097/JOM.0b013e3182307845 PMID:22068131

Park JS, Kwak SJ, Gil HW, Kim SY, Hong SY (2013). Glufosinate herbicide intoxication causing unconsciousness, convulsion, and 6th cranial nerve palsy. *J Korean Med Sci*, 28(11):1687–9. doi:10.3346/jkms.2013.28.11.1687 PMID:24265537

Paz-y-Miño C, Muñoz MJ, Maldonado A, Valladares C, Cumbal N, Herrera C *et al.* (2011). Baseline determination in social, health, and genetic areas in communities affected by glyphosate aerial spraying on the northeastern Ecuadorian border. *Rev Environ Health*, 26(1):45–51. doi:10.1515/reveh.2011.007 PMID:21714381

Paz-y-Miño C, Sánchez ME, Aréval M, Muñoz MJ, Witte T, De-la-Carrera GO *et al.* (2007). Evaluation of DNA damage in an Ecuadorian population exposed to glyphosate. *Genet Mol Biol*, 30(2):456–60. doi:10.1590/S1415-47572007000300026

Peluso M, Munnia A, Bolognesi C, Parodi S (1998). ^{32}P-postlabeling detection of DNA adducts in mice treated with the herbicide Roundup. *Environ Mol Mutagen*, 31(1):55–9. doi:10.1002/(SICI)1098-2280(1998)31:1<55::AID-EM8>3.0.CO;2-A PMID:9464316

Perry L, Adams RD, Bennett AR, Lupton DJ, Jackson G, Good AM *et al.* (2014). National toxicovigilance for pesticide exposures resulting in health care contact - An example from the UK's National Poisons Information Service. *Clin Toxicol (Phila)*, 52(5):549–55. doi:10.3109/15563650.2014.908203 PMID:24735003

Pesticide Residues Committee (2007). Pesticide residues monitoring report. Fourth quarter report 2006. York: Pesticide Residues Committee. Available from: http://www.pesticides.gov.uk/guidance/industries/pesticides/advisory-groups/PRiF/PRC-Pesticides-Residues-Commitee/PRC_Results_and_Reports/PRC_Reports_by_Year/pesticide-residue-committee-prc-2006, accessed 2 November 2014.

Pesticide Residues Committee (2008). Pesticide residues monitoring report. Fourth quarter report 2007. York: Pesticide Residues Committee. Available from: http://www.pesticides.gov.uk/guidance/industries/pesticides/advisory-groups/PRiF/PRC-Pesticides-Residues-Commitee/PRC_Results_and_Reports/PRC_Reports_by_Year/pesticides-residues-committee-prc-reports-2007, accessed 2 November 2014.

Pesticide Residues Committee (2009). Pesticide residues monitoring report. Fourth quarter report 2008. York: Pesticide Residues Committee. Available from: http://www.pesticides.gov.uk/guidance/industries/pesticides/advisory-groups/PRiF/PRC-Pesticides-Residues-Commitee/PRC_Results_and_Reports/PRC_Reports_by_Year/pesticide-residues-committee-prc-reports-2009.htm?wbc_purpose=Ba, accessed 2 November 2014.

Pesticide Residues Committee (2010). Pesticide residues monitoring report. Fourth quarter report 2009. York: Pesticide Residues Committee. Available from: http://www.pesticides.gov.uk/guidance/industries/pesticides/advisory-groups/PRiF/PRC-Pesticides-Residues-Commitee/PRC_Results_and_Reports/PRC_Reports_by_Year/pesticide-residues-committee-prc-reports-2010, accessed 2 November 2014.

Piola L, Fuchs J, Oneto ML, Basack S, Kesten E, Casabé N (2013). Comparative toxicity of two glyphosate-based formulations to *Eisenia andrei* under laboratory conditions. *Chemosphere*, 91(4):545–51. doi:10.1016/j.chemosphere.2012.12.036 PMID:23332878

Poletta GL, Kleinsorge E, Paonessa A, Mudry MD, Larriera A, Siroski PA (2011). Genetic, enzymatic and developmental alterations observed in *Caiman latirostris* exposed in ovo to pesticide formulations and mixtures in an experiment simulating environmental exposure. *Ecotoxicol Environ Saf*, 74(4):852–9. doi:10.1016/j.ecoenv.2010.12.005 PMID:21185601

Poletta GL, Larriera A, Kleinsorge E, Mudry MD (2009). Genotoxicity of the herbicide formulation Roundup (glyphosate) in broad-snouted caiman (*Caiman latirostris*) evidenced by the Comet assay and the Micronucleus test. *Mutat Res*, 672(2):95–102. doi:10.1016/j.mrgentox.2008.10.007 PMID:19022394

Prasad S, Srivastava S, Singh M, Shukla Y (2009). Clastogenic effects of glyphosate in bone marrow cells of swiss albino mice. *J Toxicol*, 2009:308985 doi:10.1155/2009/308985 PMID:20107585

Rank J, Jensen AG, Skov B, Pedersen LH, Jensen K (1993). Genotoxicity testing of the herbicide Roundup and its active ingredient glyphosate isopropylamine using the mouse bone marrow micronucleus test, *Salmonella* mutagenicity test, and *Allium* anaphase-telophase test. *Mutat Res*, 300(1):29–36. doi:10.1016/0165-1218(93)90136-2 PMID:7683765

República de El Salvador (2013). Asamblea Legislativa aprueba reformas que prohíben pesticidas que dañan la salud, 5 September 2013. Available from: http://www.asamblea.gob.sv/noticias/archivo-de-noticias/asamblea-legislativa-aprueba-reformas-que-prohiben-pesticidas-que-danan-la-salud, accessed 28 April 2015. [Spanish]

Richard S, Moslemi S, Sipahutar H, Benachour N, Seralini GE (2005). Differential effects of glyphosate and Roundup on human placental cells and aromatase. *Environ Health Perspect*, 113(6):716–20. doi:10.1289/ehp.7728 PMID:15929894

Roberts DM, Buckley NA, Mohamed F, Eddleston M, Goldstein DA, Mehrsheikh A *et al.* (2010). A prospective observational study of the clinical toxicology of glyphosate-containing herbicides in adults with acute self-poisoning. *Clin Toxicol (Phila)*, 48(2):129–36. doi:10.3109/15563650903476491 PMID:20136481

Roustan A, Aye M, De Meo M, Di Giorgio C (2014). Genotoxicity of mixtures of glyphosate and atrazine and their environmental transformation products before and after photoactivation. *Chemosphere*, 108:93–100. doi:10.1016/j.chemosphere.2014.02.079 PMID:24875917

Ruder AM, Waters MA, Butler MA, Carreón T, Calvert GM, Davis-King KE *et al.*; Brain Cancer Collaborative Study Group(2004). Gliomas and farm pesticide exposure in men: the Upper Midwest Health Study. *Arch Environ Health*, 59(12):650–7. doi:10.1080/00039890409602949 PMID:16789473

Rueppel ML, Brightwell BB, Schaefer J, Marvel JT (1977). Metabolism and degradation of glyphosphate in soil and water. *J Agric Food Chem*, 25(3):517–28. doi:10.1021/jf60211a018 PMID:858844

Rumack BH (2015). Emergency medical treatment. Glyphosate isopropylamine salt. POISINDEX(R) Information System. CCIS Volume 164, edition expires May, 2015. Available from: http://toxnet.nlm.nih.gov/cgi-bin/sis/search2/f?./temp/~M2Dk5e:2.

Sanchís J, Kantiani L, Llorca M, Rubio F, Ginebreda A, Fraile J *et al.* (2012). Determination of glyphosate in groundwater samples using an ultrasensitive immunoassay and confirmation by on-line solid-phase extraction followed by liquid chromatography coupled to tandem mass spectrometry. *Anal Bioanal Chem*, 402(7):2335–45. doi:10.1007/s00216-011-5541-y PMID:22101424

Schinasi L, Leon ME (2014). Non-Hodgkin lymphoma and occupational exposure to agricultural pesticide chemical groups and active ingredients: a systematic review and meta-analysis. *Int J Environ Res Public Health*, 11(4):4449–527. doi:10.3390/ijerph110404449 PMID:24762670

Séralini GE, Clair E, Mesnage R, Gress S, Defarge N, Manuela Malatesta M *et al.* (2014). Republished study:long-term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize. *Environmental Sciences Europe*, 26(1):1–14. doi:10.1186/s12302-014-0014-5

Siddiqui S, Meghvansi MK, Khan SS (2012). Glyphosate, alachor and maleic hydrazide have genotoxic effect on *Trigonella foenum-graecum* L. *Bull Environ Contam Toxicol*, 88(5):659–65. doi:10.1007/s00128-012-0570-6 PMID:22392005

Simonsen L, Fomsgaard IS, Svensmark B, Spliid NH (2008). Fate and availability of glyphosate and AMPA in agricultural soil. *J Environ Sci Health B*, 43(5):365–75. doi:10.1080/03601230802062000 PMID:18576216

Sinhorin VD, Sinhorin AP, Teixeira JM, Miléski KM, Hansen PC, Moreira PS *et al.* (2014). Effects of the acute exposition to glyphosate-based herbicide on oxidative stress parameters and antioxidant responses in a hybrid Amazon fish surubim (*Pseudoplatystoma* sp). *Ecotoxicol Environ Saf*, 106:181–7. doi:10.1016/j.ecoenv.2014.04.040 PMID:24840881

Siviková K, Dianovský J (2006). Cytogenetic effect of technical glyphosate on cultivated bovine peripheral lymphocytes *Int J Hyg Environ Health*, 209(1):15–20. doi:10.1016/j.ijheh.2005.07.005 PMID:16373198

Slaninova A, Smutna M, Modra H, Svobodova Z (2009). A review: oxidative stress in fish induced by pesticides. *Neuro Endocrinol Lett*, 30:Suppl 1: 2–12. PMID:20027135

Solomon KR, Anadón A, Carrasquilla G, Cerdeira AL, Marshall J, Sanin LH (2007). Coca and poppy eradication in Colombia: environmental and human health assessment of aerially applied glyphosate. *Rev Environ Contam Toxicol*, 190:43–125. doi:10.1007/978-0-387-36903-7_2 PMID:17432331

Sorahan T (2015). Multiple myeloma and glyphosate use: a re-analysis of US Agricultural Health Study (AHS) data. *Int J Environ Res Public Health*, 12(2):1548–59. doi:10.3390/ijerph120201548 PMID:25635915

Sørensen FW, Gregersen M (1999). Rapid lethal intoxication caused by the herbicide glyphosate-trimesium (Touchdown). *Hum Exp Toxicol*, 18(12):735–7. doi:10.1191/096032799678839590 PMID:10627661

Sribanditmongkol P, Jutavijittum P, Pongraveevongsa P, Wunnapuk K, Durongkadech P (2012). Pathological and toxicological findings in glyphosate-surfactant herbicide fatality: a case report. *Am J Forensic Med Pathol*, 33(3):234–7. doi:10.1097/PAF.0b013e31824b936c PMID:22835958

Stella J, Ryan M (2004). Glyphosate herbicide formulation: a potentially lethal ingestion. *Emerg Med Australas*, 16(3):235–9. doi:10.1111/j.1742-6723.2004.00593.x PMID:15228468

Székács A, Darvas B (2012). Forty years with glyphosate. In: Hasaneen MNAE-G, editor. Herbicides – properties, synthesis and control of weeds. Croatia: InTech, pp. 247–84. Available from: http://cdn.intechweb.org/pdfs/25624.pdf, accessed 28 July 2015.

Takeuchi S, Iida M, Yabushita H, Matsuda T, Kojima H (2008). In vitro screening for aryl hydrocarbon receptor agonistic activity in 200 pesticides using a highly sensitive reporter cell line, DR-EcoScreen cells, and in vivo mouse liver cytochrome P450-1A induction by propanil, diuron and linuron. *Chemosphere*, 74(1):155–65. doi:10.1016/j.chemosphere.2008.08.015 PMID:18835618

Temple WA, Smith NA (1992). Glyphosate herbicide poisoning experience in New Zealand. *N Z Med J*, 105(933):173–4. PMID:1589162

Thongprakaisang S, Thiantanawat A, Rangkadilok N, Suriyo T, Satayavivad J (2013). Glyphosate induces human breast cancer cells growth via estrogen receptors. *Food Chem Toxicol*, 59:129–36. doi:10.1016/j.fct.2013.05.057 PMID:23756170

Tian J, Shi H, Li X, Yin Y, Chen L (2012). Coupling mass balance analysis and multi-criteria ranking to assess the commercial-scale synthetic alternatives: a case study on glyphosate. *Green Chem*, 14:1990–2000.

Tice RR, Austin CP, Kavlock RJ, Bucher JR (2013). Improving the human hazard characterization of chemicals: a Tox21 update. *Environ Health Perspect*, 121(7):756–65. doi:10.1289/ehp.1205784 PMID:23603828

Tomlin CDS, editor (2000). The pesticide manual: a world compendium. 12th ed. Croydon: British Crop Protection Council. Available from: http://trove.nla.gov.au/work/6273016, accessed 28 July 2015.

Transparency Market Research (2014). Global glyphosate market expected to reach US$8.79 billion in 2019. New York: Transparency Market Research. Posted on 9 December 2014. Available from: http://www.transparencymarketresearch.com/pressrelease/glyphosate-market.htm, accessed 21 April 2015.

Truta E, Vochita G, Rosu CM, Zamfirache MM, Olteanu Z (2011). Evaluation of Roundup-induced toxicity on genetic material and on length growth of barley seedlings. *Acta Biol Hung*, 62(3):290–301. doi:10.1556/ABiol.62.2011.3.8 PMID:21840831

Tu M, Hurd C, Randall JM (2001). Weed control methods handbook: tools & techniques for use in natural areas. Version April 2001. Arlington (VA): Wildland Invasive Species Team, The Nature Conservancy. Available from: http://www.invasive.org/gist/products/handbook/01.TitleContents.pdf, accessed 28 July 2015.

Uren Webster TM, Laing LV, Florance H, Santos EM (2014). Effects of glyphosate and its formulation, Roundup, on reproduction in zebrafish (*Danio rerio*). *Environ Sci Technol*, 48(2):1271–9. doi:10.1021/es404258h PMID:24364672

Vainio H, Linnainmaa K, Kähönen M, Nickels J, Hietanen E, Marniemi J *et al.* (1983). Hypolipidemia and peroxisome proliferation induced by phenoxyacetic acid herbicides in rats. *Biochem Pharmacol*, 32(18):2775–9. doi:10.1016/0006-2952(83)90091-6 PMID:6626247

Varona M, Henao GL, Díaz S, Lancheros A, Murcia A, Rodríguez N *et al.* (2009). Evaluación de los efectos del glifosato y otros plaguicidas en la salud humana en zonas objeto del programa de erradicación de cultivos ilícitos. [Effects of aerial applications of the herbicide glyphosate and insecticides on human health] *Biomedica*, 29(3):456–75. [Spanish]. doi:10.7705/biomedica.v29i3.16 PMID:20436997

Vasiluk L, Pinto LJ, Moore MM (2005). Oral bioavailability of glyphosate: studies using two intestinal cell lines. *Environ Toxicol Chem*, 24(1):153–60. doi:10.1897/04-088R.1 PMID:15683179

Vera-Candioti J, Soloneski S, Larramendy ML (2013). Evaluation of the genotoxic and cytotoxic effects of glyphosate-based herbicides in the ten spotted live-bearer fish *Cnesterodon decemmaculatus* (Jenyns, 1842). *Ecotoxicol Environ Saf*, 89:166–73. doi:10.1016/j.ecoenv.2012.11.028 PMID:23273868

Vigfusson NV, Vyse ER (1980). The effect of the pesticides, Dexon, Captan and Roundup, on sister-chromatid exchanges in human lymphocytes in vitro. *Mutat Res*, 79(1):53–7. doi:10.1016/0165-1218(80)90147-0 PMID:7432366

Waddell BL, Zahm SH, Baris D, Weisenburger DD, Holmes F, Burmeister LF *et al.* (2001). Agricultural use of organophosphate pesticides and the risk of non-Hodgkin's lymphoma among male farmers (United States). *Cancer Causes Control*, 12(6):509–17. doi:10.1023/A:1011293208949 PMID:11519759

Walsh LP, McCormick C, Martin C, Stocco DM (2000). Roundup inhibits steroidogenesis by disrupting steroidogenic acute regulatory (StAR) protein expression.

Environ Health Perspect, 108(8):769–76. doi:10.1289/ehp.00108769 PMID:10964798

Wang G, Deng S, Li C, Liu Y, Chen L, Hu C (2012). Damage to DNA caused by UV-B radiation in the desert cyanobacterium *Scytonema javanicum* and the effects of exogenous chemicals on the process. *Chemosphere*, 88(4):413–7. doi:10.1016/j.chemosphere.2012.02.056 PMID:22436589

Wester RC, Melendres J, Sarason R, McMaster J, Maibach HI (1991). Glyphosate skin binding, absorption, residual tissue distribution, and skin decontamination. *Fundam Appl Toxicol*, 16(4):725–32. doi:10.1016/0272-0590(91)90158-Z PMID:1884912

Xie L, Thrippleton K, Irwin MA, Siemering GS, Mekebri A, Crane D *et al.* (2005). Evaluation of estrogenic activities of aquatic herbicides and surfactants using an rainbow trout vitellogenin assay. *Toxicol Sci*, 87(2):391–8. doi:10.1093/toxsci/kfi249 PMID:16049272

Yadav SS, Giri S, Singha U, Boro F, Giri A (2013). Toxic and genotoxic effects of Roundup on tadpoles of the Indian skittering frog (*Euflictis cyanophlyctis*) in the presence and absence of predator stress. *Aquat Toxicol*, 132–133:1–8. doi:10.1016/j.aquatox.2013.01.016 PMID:23454306

Yin G (2011). Glyphosate: There is no substitute. Farm Chemicals International. 3 March 2011. Willoughby (OH): Meister Media Worldwide. Available from: http://www.farmchemicalsinternational.com/crop-inputs/herbicides/glyphosate-there-is-no-substitute/, accessed June 2015.

Yoshioka N, Asano M, Kuse A, Mitsuhashi T, Nagasaki Y, Ueno Y (2011). Rapid determination of glyphosate, glufosinate, bialaphos, and their major metabolites in serum by liquid chromatography-tandem mass spectrometry using hydrophilic interaction chromatography. *J Chromatogr A*, 1218(23):3675–80. doi:10.1016/j.chroma.2011.04.021 PMID:21530973

Yue Y, Zhang Y, Zhou L, Qin J, Chen X (2008). In vitro study on the binding of herbicide glyphosate to human serum albumin by optical spectroscopy and molecular modeling. *J Photochem Photobiol B*, 90(1):26–32. doi:10.1016/j.jphotobiol.2007.10.003 PMID:18035550

Zahm SH, Weisenburger DD, Babbitt PA, Saal RC, Vaught JB, Cantor KP *et al.* (1990). A case-control study of non-Hodgkin's lymphoma and the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) in eastern Nebraska. *Epidemiology*, 1(5):349–56. doi:10.1097/00001648-199009000-00004 PMID:2078610

Zhao W, Yu H, Zhang J, Shu L (2013). [Effects of glyphosate on apoptosis and expressions of androgen-binding protein and vimentin mRNA in mouse Sertoli cells] *Nan Fang Yi Ke Da Xue Xue Bao*, 33(11):1709–13. PMID:24273285

Zouaoui K, Dulaurent S, Gaulier JM, Moesch C, Lachâtre G (2013). Determination of glyphosate and AMPA in blood and urine from humans: about 13 cases of acute intoxication. *Forensic Sci Int*, 226(1–3):e20–5. doi:10.1016/j.forsciint.2012.12.010 PMID:23291146

A new insect weapon against broom

A new insect parasite has been released into the Mackenzie Basin and Upper Waitaki for the first time, to help control the spread of broom.
Date: 05 April 2012

A new insect parasite has been released into the Mackenzie basin and upper Waitaki for the first time, to help control the spread of broom.

The Department of Conservation oversaw the release of the gall mites at three sites last month – Jollie Stream on the eastern side of Lake Pukaki, Mt Ostler near Twizel and Otematata in the Waitaki valley – all with dense infestations of broom. The aim is to establish healthy populations of the mites, which can then be moved to other sites as needed.

Gall on broom plant from gall mite

Department of Conservation ranger Peter Willemse says the broom gall mite attacks broom plants in autumn and is expected to work in concert with three other broom-eating insects already established in the area, which target the plant in spring and summer.

“It is hoped that together the insects will weaken broom plants and reduce the spread of this introduced invasive weed,” he says.

The other broom-eating insects were also introduced into the area to help control broom. The broom psyllid, also released by DOC, targets the plant’s spring growth. The caterpillar of the twig miner moth, which probably self-introduced about 50 years ago, browses on the plant’s stems. While the broom seed beetle, released by Environment Canterbury over ten years ago, has larvae that eats the soft green broom seeds.

"These insects are already affecting the vigour of their host plants, in particular the twig miner, although a widespread reduction is some time off", says Willemse.

“This is a long term strategic move – it’s not a quick fix.

"In five years we should start to see a noticeable knock-back of broom as the pysllids and gall mites join forces with the other insects.”

The broom gall mite is native to Western Europe and was first brought into New Zealand by Landcare Research in 2006, with releases taking place two years later. The mite is host-specific and is considered very unlikely to attack other plant species.

The adult mites are so small you cannot see them with the naked eye and the best way to detect their presence is to look for the galls that form as the mites feed.

Media contact

Kiersten McKinley: ph +64 3 435 3185 or +64 027 3063012

Peter Willemse: ph +64 3 435 3271 or +64 027 6171629

Oregon Tries to Sweep Out Scotch Broom

Botany: A European native, the ornamental shrub came to the state in the late 1880s, but it has since become a noxiously invasive weed.

August 15, 1999 | JEFF BARNARD | ASSOCIATED PRESS

SUTHERLIN, Ore. — Driving around the corner of a logging road, botanist Jeanne Klein got a rude shock.

The golden yellow patch of Scotch broom where she had released a precious new bug was gone, ripped out by a U.S. Bureau of Land Management road crew that failed to recognize it as one of just a dozen sites where a new biological agent was set loose to control Oregon's most noxious weed.

"I released them right about here," said the BLM botanist, standing in the raw earth at the side of the gravel road. "I chose this site because there were no timber sales planned in here."

Chalk up another win for Scotch broom.

A symbol of the Plantagenet kings in its native Europe, the shrub with the brilliant golden blossoms came to Oregon in the late 1880s as an ornamental. Nurseries still sell it for landscaping.

But in timber plantations, pasture, meadows and power line right-of-ways, the shrub is a scourge, causing $47 million a year in damage. That puts it at the top of the list among noxious weeds in Oregon.

The greatest impacts are in timber plantations, where Scotch broom crowds out new seedlings. Along streams, it elbows aside native plants. In pastures and meadows, it chokes out grasses. Along power lines, it creates a fire hazard and makes the ground impassable.

Despite volunteers ripping it out of the ground with root wrenches, road crews grubbing it out with backhoes and timber crews hacking and spraying it with herbicides, Scotch broom has spread to 16 million of the some 20 million acres of western Oregon.

The march of Scotch broom could make a horror movie. The pods fling the seeds up to 35 feet, where they can lie dormant for 60 years, just waiting for a road crew to come along and rip up the ground, creating the disturbed soil conditions that trigger the seeds to sprout.

Mowing doesn't work unless the plant is dried out by drought. Root-wrenching is effective but too laborious to make much of a dent in the current stands.

Enter a pair of lowly insects, which love nothing more than to munch on Scotch broom seeds. Like a fussy child, they will eat nothing else, a key factor in winning approval as a biological control.

In 1983, the Oregon Department of Agriculture turned loose the first batch of a weevil--known to scientists as Apion fuscirostre--west of Salem.

Native to Europe, the bugs are harvested from broom on the East Coast, where they are well established, by whacking the bushes with a tennis racket and catching the bugs in a tarp. The adults lay their eggs on the seedpods, and the larvae eat up to 80% of the seeds on a bush. But that still leaves lots of seeds.

Now a new bug has been added to the fight. It is a tiny hairy beetle known as Bruchidius villosus, also a European native.

"We're hoping that between the two of them we can control most of the seeds," said Tim Butler, a field operations manager for Oregon's agriculture department.

Biological controls will never wipe out Scotch broom. The best hope is that they will make it just another member of the plant community, rather than a rampaging new arrival.

But the search is expensive. Approval of a new bug can take $10 million and 20 years of research.

Butler had Bruchidius villosus for only 12 sites this year, and the 250 individuals that Klein released above Hubbard Creek represented the only site in Douglas County.

Klein walked across the road to some surviving Scotch broom and shook the branches over a blue tarp, hoping to see some of her bugs. About 100 would need to survive to start a viable population. Several Apion fuscirostre fell out, and perhaps one of the Bruchidius.

"This could be one," she said, reaching for the bug just as the wind blew it away. "Whoops. That was encouraging to at least think I had one for a moment."

Host-specificity testing the French broom psyllid *Arytinnis hakani* (Loginova)

T. Thomann[1] and A.W. Sheppard[2]

[1]CSIRO European Laboratory, Campus de Baillarguet, 34980 Montferrier-sur-Lez, France
[2]CSIRO Entomology, GPO Box 1700, Canberra, ACT 2601, Australia

The broom psyllids are known to have strong co-evolutionary relationships with their related host plants. For this reason, the French broom psyllid, *Arytinnis hakani* (Loginova) (Homoptera: Psyllidae), was selected as potential biological control agent against its host plant, *Genista monspessulana* (L.) Johnson, a Mediterranean leguminous shrub invasive in Australia and California. Between 2002 and 2006, two types of host-specificity test were conducted on potted plants: (a) choice-without-target tests, which evaluated the capacity of the insect to lay eggs on test plant species in the absence of the natural host and (b) no-choice starvation tests, where the first-instar nymphs are forced to develop on test plant species other than the natural host. Over 92 species were tested in 47 genera covering ten plant families. The tests revealed that *A. hakani* can potentially develop on plant species from four genera within the Genisteae tribe (including the target), with nymphal development on species from two genera within the Thermopsidae tribe. The high number of species with nymph development in the genus *Lupinus* (16 of 25 tested) may lead us to reconsider *A. hakani* as a potential biological control agent against *G. monspessulana* in the USA. Further work on imported exotic lupines of economic importance to Australia is required to assess potential for release there.

Prospects for the biocontrol of Banana Passionfruit in New Zealand with a *Septoria* leaf pathogen

N.W. Waipara,[1] A.H. Gourlay,[2] A.F. Gianotti,[1] J. Barton,[2] L.S. Nagasawa[3] and E.M. Killgore[3]

[1]Landcare Research, Private Bag 92170, Auckland, New Zealand
[2]Landcare Research, PO Box 40, Lincoln, New Zealand
[3]Hawaii Department of Agriculture, 1428 South King Street, Honolulu, HI 96814, USA

Seven closely related vine species of *Passiflora*, all with the common name banana passion fruit and of South American origin, have naturalized and become serious environmental weeds in various regions throughout New Zealand. Banana passion fruit is capable of smothering trees, particularly those at forest margins and in forest gaps. It often prevents regeneration of native plants and has therefore been classified as a priority weed for biocontrol by invasive plant biosecurity managers in New Zealand. It is also a significant environmental threat in Hawaii where it is known as banana poka. A successful classical biological weed control programme was undertaken with the release in 1996 of a virulent leaf pathogen, *Septoria passiflorae*. A similar biological control programme was initiated in New Zealand to explore the efficacy and safety of *S. passiflorae* for its potential introduction against this rapidly expanding and hybridizing weedy complex. Pathogenicity testing showed the fungus to be a virulent pathogen against the banana passion fruit weed complex, with promising biocontrol prospects. However, its release in New Zealand may be prevented due to its potential damage to the closely related commercially cultivated species *Passiflora edulis.*