Midpeninsula Regional Open Space
Integrated Pest Management Program

Pesticide Literature Review and Annotated Bibliography

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1.1 Introduction

The management of natural areas requires that invasive species be controlled in order to minimize their impacts to the plants and animals that inhabit those areas. Midpeninsula Regional Open Space District (hereafter MROSD) actively manages invasive plants and animals that pose significant risk to plant and animal populations, communities, and ecosystem processes. MROSD employs an integrated pest management approach to minimize the negative impacts of invasive species. The aim of MROSD's integrated pest management plan is to use the most effective and least toxic treatment options to control invasive species. Minimizing risk requires an understanding of the effects of management actions on non-target organisms and the persistence of pesticides in the environment following application. Pesticides are an important component of invasive species control and are generally far more effective and less expensive ($/area) than manual control approaches (e.g. hand pulling, mowing, etc.). The use of these chemicals can themselves cause impacts to non-target organisms and human health and it is important to evaluate and understand the risks associated with pesticide use. These risks should then be weighed against the risks of not using the pesticide(s) and permitting the expansion of invasive pest species.

The information provided in this report describes the state of the knowledge regarding pesticides used by MROSD and their efficacy at controlling target species, effects on non-target organisms, and their persistence in the environment

1.2 Literature Review and Evaluation Process

I performed a comprehensive literature review of all the scientific papers published between 2015 and 2018 on the 18 active ingredients included in the MROSD Integrated Pest Management Plan (Environmental Assessment). All papers included in the review were peer reviewed and published in reputable journals and indexed in Web of Science. I completed a literature search for each of the 18 pesticides in Web of Science that included the pesticide name as the sole search term and refined each search to include only papers published from 2015 - 2018. The titles and abstracts of all papers returned by the query were read. If the topic of the paper included human health risks, acute or chronic impacts to non-target organisms (i.e. plants, animals, bacteria, or fungi), pesticide effectiveness, or persistence in the environment, then the papers were reviewed and described in an annotated bibliography (Appendix 1). All study findings described in the report and the annotated bibliography are supported by appropriate statistical analyses unless otherwise stated.

2.0 HERBICIDES

2.1 GLYPHOSATE

Glyphosate is a broad-spectrum herbicide used for its high effectiveness and low cost. Glyphosate deters plant growth by inhibiting the shikimate pathway that prevent the synthesis of aromatic amino acids. The shikimate pathway is only present in plants, fungi, and some microorganisms. Animals lack the shikimate pathway, which is why glyphosate has historically been considered one of the least toxic herbicides.

Here I describe studies published between 2015 and 2018 that have evaluated the effects of glyphosate on non-target organisms including: animals, microorganisms, and non-target plants. When examining the risks of pesticides on non-target organisms, it is important to evaluate the range of ecologically relevant concentrations. The amount of glyphosate encountered by non-target organisms depends upon the application rate, the frequency of applications, the size of the area being treated, whether the applications are direct or indirect, and the degradation rate of the chemical in the environment.

The ideal way to evaluate non-target impacts is to use a dose-response design, where by non-target organisms are exposed to a gradient of doses that range from concentrations likely encountered in the environment to doses that exceed realistic environmental concentrations. The magnitude of the response of an organism to a chemical is expected to increase with the dose. Many of the studies included in this review that employed a dose response approach found effects of herbicide at intermediate dose, but not at higher doses. These types of responses suggest that either the study organisms are able to (physiologically or otherwise) overcome the stress associated with glyphosate at higher dose rates, or that the result is spurious. Either way, these findings are not easily interpreted.

2.1.1 Exposure scenarios

Compared to agricultural uses, the use of glyphosate in natural lands management creates very different exposure risks to non-target organisms (including people). In agriculture, glyphosate use is intensive and has increased exponentially over the last two decades as a result of genetically modified crop species that are resistant to glyphosate. The use of these genetically modified crops has increased the number of glyphosate applications that can occur per year because farmers can spray weeds when crop plants are present without damaging the crops. Most studies that evaluate the risks of glyphosate are in an agricultural context and apply concentrations that far exceed the concentrations likely encountered by applicators in these glyphosate intensive agricultural scenarios.

At MROSD, glyphosate's potential to affect non-target organisms is mitigated by several procedures. First, it is only used when other non-chemical control methods (e.g. hand pulling, mowing, etc.) are not feasible or effective. The agency applies glyphosate with either hand held low pressure (30-70 psi) wands attached to backpack sprayers, or buy wiping cut stumps with glyphosate-soaked sponges. Both of these techniques are highly localized and greatly minimize glyphosate contact with non-target organisms. Lastly, MROSD also only applies glyphosate when winds are below 7mph and there is a 24 window of time with less than a 40% chance of rain in the forecast.

As of 2018, the EPA maintains that the overall weight of evidence suggests that glyphosate is safe to humans and the environment. A portion of the public is concerned about risks associated with glyphosate use. A portion of the public is also distrustful of regulatory agencies, perhaps rightfully so: there is evidence that suggests that Monsanto, the producers of glyphosate, have been steering the science and the EPA's regulatory decisions (described in Robinson et al. 2013). The enormity of Monsanto's power and influence and their documented meddling with the agency that is supposed to regulate them in the public interest have given rise to two primary public concerns: human health risks and ecological (environmental) risks.

2.1.2 Human health risks

A contentious debate surrounding the human health risks of glyphosate has far from been resolved. The large body of scientific studies on glyphosate have conflicting and complicated results and various shortcomings: most are limited by sample size, confounding factors, a lack of a dose response, or unrealistic exposure levels. Of those that demonstrate some effect, the effect is only seen at low or intermediate concentrations of glyphosate, but not at higher concentrations. This makes interpretation of study results difficult, even for experts. Other times the negative effects of glyphosate are only found when glyphosate exposure far exceed environmentally relevant concentrations

In 2015 the International Agency for Research on Cancer (IARC) classified glyphosate as "probably carcinogenic to humans," an overarching factor in this decision is a handful of studies that have found a positive association between occupational exposure to glyphosate and non-Hodgkin lymphoma (NHL) and several lab studies (on lab rats and mice) that demonstrate the mechanistic potential for this link (described in Guyton et al. 2015). At the same time several equally credible studies have not found any links between glyphosate and cancer (reviewed in Williams et al. 2016). Another fair critique in defense of the safety of glyphosate is that in most studies find that the lethal and non-lethal effects of glyphosate are not observed until well above environmentally relevant concentrations. The most extensive study concerning cancer risks associated with occupational glyphosate use is the ongoing Agricultural Health Study that includes 55,000 farmers, and over 45,000 who are exposed to glyphosate with varying frequency and intensity. The conclusions from this important study neither support or counter the argument that glyphosate is probably carcinogenic. Andreotti et al. (2018) concludes that no strong evidence was found that glyphosate use increases the risk solid tumors or lymphoid malignancies, including non-Hodgkin's lymphoma. However, Andreotti et al. (2018) did find some preliminary evidence, though not statistically significant, of a possible association between myeloid leukemia and glyphosate use when they only considered the subset of study participants that had the most intensive glyphosate exposure, that is people who used glyphosate on average 38 days/year for 8 years. Animal and human studies were evaluated by regulatory agencies in the USA, Canada, Japan, Australia, and the European Union, as well as the Joint Meeting on Pesticide Residues of the United Nations and World Health Organization. These agencies looked at cancer rates in humans and studies where laboratory animals were fed high doses of glyphosate. Based on these studies, they determined that glyphosate is not likely to be carcinogenic. Collectively these finding suggests that MROSD's applicators and the human population exposed to MROSD applications of glyphosate are seemingly safe.

While the evidence linking glyphosate to cancer is extremely weak, given the public perceptions and general uncertainty surrounding glyphosate risks, I suggest that glyphosate applicators err on the side of caution and take standard precautions to reduce exposure. Best management practices should include personal protection equipment that reduces direct contact and exposure. For example, applicators should protect skin with chemical resistant gloves, clean equipment after use, wash hand and clothing thoroughly after handling, avoid eating until after washing hands, and avoid breathing vapor or mist.

2.1.3 Ecological risks

The use of glyphosate, or restrictions of its use, should carefully consider the ecological cost associated with using or not using glyphosate to control invasive plants in natural areas. Often, the ecological cost of not using glyphosate to control invasive plants poses greater risk than the risks associated with the of use of glyphosate. Non-herbicide invasive plant control treatment (e.g. mowing, hand pulling, flaming, etc.) have been shown time and time again to be far less effective at controlling invaders. Whereas glyphosate is consistently effective at reducing populations of invasive plant species and poses less risk to non-target organisms than other comparably effective herbicides. Furthermore, the ecological risks associated with glyphosate have been studied far more frequently than other herbicides. Since 2015 over 1500 scientific papers have been published about glyphosate. This literature is an order of magnitude richer than the literature on any other herbicide used to control invasive plants at MROSD. After thoroughly reviewing the literature on the impacts of glyphosate on non-target species my overall opinion is that acute exposure to environmentally relevant concentrations of glyphosate is not toxic to animals, but that acute and chronic glyphosate exposure can have non-lethal but detrimental impacts on bees and amphibians.

Bees and Butterflies

Bees and butterflies deserve special consideration because of their crucial role as pollinators in terrestrial ecosystems. Pollinator decline over the past decade has been attributed to multiple factors including pathogens and the use of pesticides (Potts et al. 2016). Clear evidence has demonstrated the negative impacts of insecticides, particularly neonicotinoids on bees (Goulson 2013). Pollinator decline has spurred research on the effects of glyphosate on pollinator health, particularly honeybee health. Between 2015 and 2018 there were twelve papers published that evaluated the effects of glyphosate on bee survival, growth, reproduction, behavior, gut microbiome, and pathogen resistance. Bees can come into contact with pesticides by flying through herbicide spray or through contaminated dust, or by foraging on treated plants and feeding on nectar or pollen that contains glyphosate traces. These bees can then transport the contaminated pollen and nectar into hives and expose the brood.

Since MROSD does not use broadcast spray approaches to apply herbicide and instead uses hand held backpack sprayers that allow applicators to place herbicide precisely on target plants, it is highly unlikely that bees (or other flying insects) will encounter herbicide spray directly. However, bees and other pollinators could be exposed to glyphosate by foraging on treated plants and feeding on nectar or pollen that contains pesticide traces. The presence of glyphosate traces in honey samples suggests that brood come into contact with glyphosate within their colonies. In Hawaii the occurance of glyphosate in honey was predominatly in hives that are near agricultural areas and golf courses (Berg et al. 2018). Findings from this study suggest that glyphosate use associtaed with invasive species management in natural areas does not contribute to honey contamination.

In agricultural areas where fields are typically broadcast sprayed from aircraft, or booms attached to tractors or other mechanical equipment, glyphosate drift can contaminate non-target plants. Cebotari et al. (2018) measured the concentration of pesticide residues in *Robinia pseudoacacia* and *Tilia platyphillos* flowers from a forest that is near to agricultural land. The detected concentrations of glyphosate residues in the tree flowers was 1.6 to 8 times lower than the maximum limits allowed according to national and European Union standards. In natural areas that are far from agricultural areas glyphosate residue in flowers is likely negligible, though more research is needed to confirm this. Since MROSD uses hand held applicator wands or sponges herbicide drift is minimal and not likely to substantially contaminate the flowers of non-target plants which reduces the risk of pollinator exposure.

It appears that bees do not have the ability to avoid contaminated plants. Fagundez et al. (2016) evaluated honey bee visitation to soybean flowers immediately after being sprayed with glyphosate to determine if glyphosate-contaminated flowers repel bees. Bee visitation to soybean flowers was not influenced by glyphosate applications. Time following herbicide application did not influence bee visitation. These findings suggest that bees do not avoid flowers contaminated with glyphosate. A shortcoming of this study is that it did not provide bees with a choice to visit unsprayed soybean flowers or compare bee visits to soybean flowers in unsprayed fields. To determine if bees can avoid food sources contaminated with glyphosate Liao et al. (2017) conducted a feeding choice experiment and offered bees sugar water and sugar water contaminated with glyphosate. Bees preferred sugar water with 10 ppb of glyphosate over the sugar water control, but did not discriminate between the sugar water control and sugar water contaminated with higher glyphosate concentrations. These finding provide further support that bees do not discriminate against glyphosate treated food when given the choice. The glyphosate concentrations bees are likely to encounter when foraging on flowering plants following a glyphosate application in agricultural systems ranges between 1.4 and 5.2 mg/L (Herbert et al. 2014). The concentrations in flowering plants in natural areas where glyphosate is applied by hand is unknown, but is likely far lower than in agricultural systems.

The effects of glyphosate on bees that do consume pollen or nectar from contaminated plants are variable and depend upon glyphosate concentrations and whether the herbicide has dried prior to exposure. Stingless bees in Brazil that were fed Roundup contaminated food had faster mortality rates and overall higher mortality than the bees in the control group (Seide et al. 2018), however, the concentrations of Roundup fed to the bees in this study greatly exceeded environmentally relevant concentration, so it is difficult to interpret these findings in the context of natural lands management. In a cage experiment, Abraham et al. (2018) found that bees placed in immediate contact with plants that had just been sprayed with glyphosate (at the recommended application rate) had a 20% increase in mortality, but that bee mortality was not influenced by glyphosate if it was allowed to dry prior to contact. To minimize bee contact with glyphosate-wetted plants, MROSD should consider spraying flowering plants at times during the day when bees are less active or before or after peak bloom.

Recent studies that examined the effects of glyphosate on bee development and morphology have found mixed results. Dai et al. (2018) fed bee larvae a sugar solution spiked with 0.8, 4, or 20 mg/L of glyphosate and found that regardless of the concentration, neither larval development rate or body size were affected by glyphosate.

Royal jelly is synthesized in the hypopharyngeal glands of bees and is an essential protein for feeding the colony, especially the queen. Glyphosate had no effect on the amount of royal jelly produced by nurse bees or the proportion of royal jelly consumed by larvae (Faita et al. 2018). Mengoni Gonalons and Farina (2018) also exposed honey bees to a diet that contained glyphosate and found that bee mortality was not affected by glyphosate, but that in 1 of 3 trials bees consumed less food when it was contaminated with glyphosate.

The most significant study published between 2015 and 2018 regarding glyphosate exposure and bee health examined the effects of glyphosate on the microbiome in guts of honey bees. In this elegant study, Motta et al. (2018) found that the abundance of the 8 most important bacterial species in bee guts declined substantially following glyphosate exposure. These gut bacteria produce an enzyme that is known to promote weight gain and reduce pathogen susceptibility. Motta et al. (2018) further found that bees exposed to glyphosate are more susceptible to infection by a particular pathogen, *Serratia marcescens*. While this study does provide compelling evidence that glyphosate can negatively impact the microbiome of bees, the implications that this has on population viability remains unknown. Further studies are needed to understand if gut microbiome dysbiosis is the cause of increased *Serratia marcescens* infection. *Serratia marcescens* is currently considered an innocuous pathogen that does not have known impacts on bee health. It will be important to know if infection by pathogens that do have population level consequences for bees also increase as a result of glyphosate exposure.

Balbuena et al. (2015) measured the effects of 2.5, 5, and 10 mg/L of glyphosate in a sugar solution on bee flight patterns. They measured the proportion of bees that flew directly to the hive compared to the proportion that took indirect flight paths. They also compared flight times between treated bees and control bees. They found that bees exposed to 10 mg/L of glyphosate contaminated food had longer flight times when taking the direct path, but that the lower doses had no effect on flight time. These findings suggest that environmentally relevant concentrations of glyphosate do not impair flight time or flight patterns, but that glyphosate applied at 3x the recommended application rate can have negative impacts on bee flight.

Only one study was published between 2015 and 2018 evaluated the risk of butterfly health and glyphosate exposure. Saunders et al. (2018) estimated several factors such as climate, water availability, land cover type, and county level glyphosate application from 1994-2012 to examine potential factors contributing to Monarch butterfly decline in the Midwest (USA). The found a negative relationship between monarch abundance and county level glyphosate use on corn and soybean crops, suggesting that glyphosate and the abundance of land in the Midwest used for growing glyphosate resistant corn and soybean are contributing factors to population declines of Monarch butterflies.

These studies collectively suggest that concentrations of glyphosate that pollinators are likely to encounter following glyphosate treatments at MROSD do not pose substantial risk to the viability of bee or butterfly populations. Furthermore, it is far more plausible that allowing invasive species to persist and spread will have a greater negative effect on bees and other pollinators than cautious use of glyphosate. Invasive species are frequently able to self-pollinate or reproduce asexually and therefore provide less pollen and nectar rewards to pollinators. Theoretically this could result in declines in pollinator abundance, which could in turn reduce pollination success of flowering native plants that require outcrossing for reproduction. For invasive plants that do provide large pollen and nectar rewards, the dependence pollinators have on native plants may decrease, with negative consequences for native plants that depend on pollinators for reproduction.

Amphibians

Glyphosate's potential effect on amphibians is of special concern because amphibians are declining globally (Hoffmann et al. 2010, Hof et al. 2011) and their life histories, behaviors, and morphologies all make them especially vulnerable to potential environmental toxins. Amphibians exchange gases through highly permeable skin, have low vagility, and life cycles tied to surface waters. They also depend on both aquatic and terrestrial environments at different stages of their life cycle and have different diets as larvae (tadpoles) than as adults.

Amphibians are probably most vulnerable to glyphosate exposure during the aquatic phase of their lifecycles, and MROSD's current practices minimize the amount of glyphosate amphibians are likely to encounter in their aquatic habitats. The amount of glyphosate present in aquatic habitats depends on application rates, interception by vegetation, and whether the applications are direct or indirect. MROSD only applies glyphosate directly onto vegetation when rain is unlikely to rinse glyphosate off of plants and into soil or water. These application methods greatly reduce glyphosate exposure to all aquatic organisms, amphibians included.

There were 25 papers published between 2015 and 2018 that evaluated the effects of glyphosate on amphibian mortality, morphological development, physiology, reproduction, behavior, and pathogen susceptibility. Each of these studies are described in the annotated bibliography (Appendix 1). Studies that determined amphibian LC50 values between 2015 and 2018 are presented in Table 1. Of these 25 studies, only Vincent and Davidson (2015) considered amphibian taxa present in the general vicinity of MROSD's jurisdiction. Vincent and Davidson (2015) determined the toxicity of Aquamaster (now called Roundup custom) on Western toad tadpoles in the San Francisco Bay area were several orders of magnitude above estimated environmental concentrations allowed by the EPA (3.72 mg/L), suggesting local current glyphosate application practices are adequately cautious (LC50 values presented in Table 1).

Nine studies assessed the effect of environmentally relevant concentrations of glyphosate on amphibian mortality between 2015 and 2019. Of these, eight found that glyphosate does not result in acute toxicity (Levis and Johnson 2015, Ujszegi et al. 2015, Vincent and Davidson 2015, Baier et al. 2016, Dornelles and Oliveira 2016, Krynak et al. 2017, Boone 2018, Relyea 2018). However, Krynak et al. (2017) found that 2.5 mg/L of a glyphosate-based herbicide, reduced survival of Blanchard's cricket frog tadpoles by 37%. While this finding appears to be an outlier, it is an important one because the mortality effect is substantial while the dosage is within the environmentally relevant range, though probably higher than concentrations encountered in MROSD treated habitats. The only study that evaluated glyphosate effects on amphibian embryos used concentrations that far exceeded environmentally relevant concentrations, and only reported increased mortality of Mediterranean painted frog embryos following exposure to their highest two experimental concentrations of Roundup (180 mg/L and 225 mg/L).

While not lethal, disruption of growth patterns has profound consequences for amphibian populations. Like most animals, disruptions in growth patterns can affect amphibian's ability to migrate, hunt, and evade predators. Because amphibians lay eggs, female size is closely linked to fecundity, so growth disruptions can have demographic consequences tantamount to mortality. Fortunately, like mortality, amphibian growth was rarely impacted by glyphosate-based herbicides. Out of 9 studies published between 2015-2018, 7 studies found that tadpole mortality was not associated with glyphosate exposure (Baier et al. 2016, Costa and Nomura 2015, Dornelles and Oliveira 2016, Krynak et al. 2017, Miko et al. 2015, Soloneski et al. 2016, Ujszegi et al. 2015). African clawed frog tadpoles exposed to 4.5 and 9 mg/L were smaller than the controls, but higher doses had no effect (Wagner et al. 2017). The relevance of this solitary negative finding to MROSD is minimal for two reasons. First, all doses used in this study are well above any concentrations of glyphosate that might be generated by MROSD activities. Second, the lack of a response at higher doses (much higher than would be used by

MROSD) suggests a spurious result, and complicates any interpretation of these results. The only other study documenting an effect on amphibian growth found reduced body size of Agile frog tadpoles exposed to 2 mg/L of glyphosate in the lab, but increased body size in and outdoor mesocosm (Miko et al. 2015). This study is important because laboratory studies on pesticides are far more common than field studies, yet this work suggests that effects of glyphosate may completely reverse in more natural real-world contexts. To date much of our understanding regarding pesticide impacts on amphibians come from lab experiments, yet the application of pesticides is almost always in the field.

Like growth disruption, DNA alteration is a serious potential consequence of exposure to pesticides for any organism. Amphibians aquatic life histories and permeable skins make them potentially especially vulnerable to this threat because they make it more likely that potentially mutagenetic agents actually come into contact with genetic material. Chemicals that bind to DNA can result in DNA breakage, the loss of genetic material, sister chromatid exchanges, and can trigger carcinogenic processes. Unfortunately, there are few recent studies on the effects of glyphosate on amphibian genetic material, and those that exist suggest cause for concern. Soloneski et al. (2016) found glyphosate exposure increased DNA damage in *Rhinella arenarum* toad cells by 55%. Carvalho et al. (2018) reported DNA damage in *Dendropssophus minutus* frog egg masses at only the lowest concentration (0.28 mg/L) in a series of experimental exposures of environmentally relevant concentrations of Roundup. As observed in several other studies on glyphosate, the lack of a response at higher concentrations impedes interpretation and suggests a spurious result. When taken together, these two studies indicate a possible negative effect of glyphosate on amphibian DNA, even at the low environmental concentrations that might be typical to MRSOD activities. However, the existing evidence is slim and its relevance to real world applications is dubious. The clearest message generated by these studies is that more work on glyphosate’s effect on amphibian DNA is urgently needed.

Amphibians are unique in having permeable skin that is used in gas exchange. As such, studies that consider glyphosate’s effect on skin morphology and function are especially valuable. Rissoli et al. (2016) compared the effects of two different glyphosate-based pesticides

(Roundup Original and Transorb) and pure glyphosate on two closely-linked and vital aspects of amphibian physiology: skin and metabolic rate. They found that Roundup Original and glyphosate both increased skin thickness, only glyphosate reduced metabolic rate, and only Transorb increase metabolic rate. These results suggest that the surfactants and inert chemicals present in various commercial formulations of glyphosate may affect how glyphosate affects amphibian physiology. Krynak et al. (2017) found that 2.5 mg/L of glyphosate changed the bacterial community composition on the skin of Blanchard's Cricket Frog tadpoles (*Acris blanchardi)*, but that lower glyphosate concentrations did not have this effect, and this effect did not persist through metamorphosis. While this study reveals a potentially important effect of glyphosate on amphibians, the authors could not connect it to decreased amphibian survival or to any of the disease states associated with amphibian decline.

Stress is now widely recognized as an important sublethal effect among wildlife and conservation biologists, and several papers on glyphosates and amphibians published between 2015 and 2018 reflect this trend. Many physiological details of stress response are broadly similar across a wide range of taxa, meaning that stress can be easily detected in organisms, and stress response can implicate other physiological disturbances in organisms, even when the underlying mechanisms are unknown. Finally, it is widely accepted that chronic stress has individual fitness and population-level consequences. Burraco and Gomez-Mestre (2016) evaluated a variety of stress indicators in spadefoot toad tadpoles exposed to low (1 mg/L) and high (2mg/L) concentrations of glyphosate for 10 days and obtained contradictory results: corticosterone production increased 91% at low concentrations (1 mg/L) but not at high concentrations (2mg/L). Both concentrations more than doubled metabolic rate, but had no effect on immune function or antioxidant enzyme activity. This result clearly suggests that glyphosate can affect amphibian metabolic rate, but all other conclusions are murky and highlight the need for more detailed studies on glyphosate's effect on amphibian stress. Levis and Johnson (2015) found that moderate light levels interacted with 3mg/L glyphosate exposure to decrease spotted salamander tadpole immune response, but not at low light levels. This finding is noteworthy because immune system suppression is a hallmark of stress response and because few studies consider possible interactions between glyphosate and environmental factors like light exposure.

Several authors who find sublethal effects of glyphosate on amphibians suggest that the effect they observe may be caused by an underlying stress response. Linking sublethal effects to stress implies that these sublethal effects are likely to have important consequences for the affected amphibians. However, for many of these assertions the link to stress response has not been compellingly demonstrated. Costa and Nomura (2016) suggest that the nose and snout asymmetry they document in *Physalaemus cuvieri* frog tadpoles exposed to glyphosate is an indicator of environmental stress. Similarly, Dornelles and Oliveira (2016) found that bullfrogs exposed to Roundup had lower glycogen and lipid concentrations, consistent with a stress response. Levi and Johnson (2015) suggest that the observed increase in metamorphosis rate of spotted salamander tadpoles in the presence of 3 mg/L of glyphosate at various light exposures indicates that the herbicide may trigger a stress response. In all these cases, more work is needed to demonstrate a clear link between these effects, stress, individual survival, and amphibian population changes.

Several authors document sublethal effects of glyphosate on amphibians that have unclear implications for amphibian health. Some document a clear response to glyphosate, but fail to link the response to a biologically relevant consequence. In some cases, the survival consequences of the effect are clear, but the relationship between glyphosate concentration and the effect defies interpretation or implementation for MRSOD land managers.

Bach et al. (2018) compared the effects of Roundup Ultramax and pure glyphosate ranging from environmentally relevant (0.37 mg/L) to extremely high (300mg/L) concentrations on the microscopic cell structure (histology) in tadpole livers of the neotropical frog, *Leptodactylus latrans*. This comparison was designed to distinguish the effects of pure glyphosate from glyphosate mixed with other chemicals found in the Roundup Ultramax formulation. They report increased changes in liver cell structure with only the lowest concentration of Roundup (0.37 mg/L), and the highest two concentrations of pure glyphosate

(75 mg/L and 300 mg/L). These results for Roundup are not actionable because higher doses showed no effect, while the results for glyphosate are far above concentrations that MRSOD would be using. They also found increased hepatic lesions for intermediate doses of both herbicides. Again, and as with many other studies, the absence of a clear relationship between concentration and effect are an impediment to drawing broader conclusions. Interestingly, the authors speculate that low concentrations might trigger a hormonal response prompting the liver to detoxify glyphosate that is not triggered at higher concentrations. While the authors suggest a mechanism, it raises more questions than answers: they do not suggest why high glyphosate concentrations wouldn't trigger a similar hormonal response.

Intermediate concentrations of glyphosate shortened tail lengths and increased the frequency of tail curvature in Common toad (*Bufo bufo*) tadpoles (Baier et al. 2016). As in many studies, the lack of an effect at low or high glyphosate concentrations severely limits the application of this study to other contexts. Additionally, there is little known about the consequences of shortened tail lengths or tail curvature. Shorter tails may reduce the speed that tadpoles can move to escape predation, but further studies are needed to confirm the relationship between tail length and agility.

Ujszegi et al. (2015) evaluated survival, behavior, body mass and predatory activity of smooth newts exposed to 6.5 mg/L of Glyphogan Classic, a glyphosate-based herbicide widely used in Europe. No differences in survival, behavior, body mass and predatory activity were detected between newts exposed to glyphosate and the control.

Recent work done on glyphosate's effect on amphibians highlights the great need for studies more applicable to field settings. Some mesocosm experiments have results that directly oppose patterns indicated in laboratory studies. Several studies published between 2015 and 2018 are starting to bridge the gap between laboratory findings and field applications by using mesocosms, using semi-natural experimental designs, or considering the interactions other factors may have on glyphosate's effect on amphibians. While these efforts are necessary and important, thus far the results are difficult to draw actionable conclusions from. One of the most intriguing results in this realm finds that Agile frog tadpoles exposed to glyphosate grew larger relative to controls in outdoor mesocosms, but smaller in laboratory conditions with the same glyphosate treatment (Miko et al. 2015). The authors do not suggest a mechanism for this pattern reversal, but the result highlights the limited applicability of laboratory studies for land managers.

Bokony et al. (2017) used two separate experiments to evaluate the relationship between glyphosate concentration, exposure duration, and the production of an important predator-defense compound produced by common toad tadpoles. Only tadpoles that were exposed to herbicide the entire duration of the study produced increased concentrations of the defensive compound. They also demonstrated a clear relationship between glyphosate concertation and the toad defense compound. While this study is exceptional in both the relevance of its approach, and the clarity of its results, the ecological implications of increased defense chemical production is unknown. Increased larval chemical defense might reduce predation, but alternatively could make larva more vulnerable to predation by invertebrates if the chemical production reduces development and growth. Gungordu et al. (2016) evaluated the effects of interactions between five glyphosate concentrations ranging from 0.8 mg/L to 12.8 mg/L and another (methidathion-based) pesticide on three frog species. They measured the activity of six different biochemical enzymes but found no clear effects. Furthermore, they do not address how these enzymes contribute to frog survival or fitness.

Moore et al. (2015) examined how glyphosate interacts with the presence or absence of amphibians' predators and found that wood frog tadpoles exposed to glyphosate did not reduce movement when exposed to predator chemical cues. This study suggests glyphosate suppresses an important behavioral response in these amphibians. Relyea (2018) also investigated the effect of interactions between predators, predator chemical cues, and glyphosate on mortality in several frog species in outdoor mesocosms. Unfortunately, all treatments with predators resulted in 100% mortality, precluding any useful broader conclusions regarding glyphosate.

Amphibians are vulnerable to UV radiation due to their unprotected eggs and thin skin. Levi and Johnson (2015) evaluated the interacting effects of low and moderate light availability and glyphosate on spotted salamander tadpole survival. While survival was not affected by glyphosate in either light condition, the proportion of tadpoles that metamorphosed increased with herbicide exposure under both light regimes. Glyphosate also appeared to suppress immune response under moderate light conditions, but had no effect under low light. Increased metamorphosis and reduced immune response both imply increased tadpole stress, so this study demonstrates both a negative effect of glyphosate alone and a negative interaction between glyphosate and increased light exposure.

Most new research agrees with past research to indicate that glyphosate has little or no clear effect on amphibian viability, despite their unique sensitivity to environmental toxins. Furthermore, when amphibians are affected, the consequences for amphibian survival and fecundity seem minimal or at worst unclear. However, it is probably prudent to minimize amphibian exposure to glyphosate for two reasons. First, many studies document an effect at some intermediate dose of glyphosate. Second, some studies indicate that glyphosate may have unpredictable interactions with other environmental factors that may affect amphibians. Together, these observations highlight the rudimentary nature of our understanding of glyphosate’s effects on amphibians. Future work must determine if effects from mid-range concentrations of glyphosate are spurious, and, if not, what mechanisms are causing effects on amphibians at only one specific dose. Additionally, recent studies make it clear that the applicability of laboratory studies to field applications of glyphosate cannot be assumed for amphibians. Future work must attempt to replicate laboratory findings under more natural conditions.

Despite the unique potential problems associated with glyphosate use around amphibians, the best evidence to emerge from 2015 to 2018 suggests that MROSD’s current practices regarding glyphosate use are extremely unlikely to have any effect on amphibians. This recent body of research certainly suggests that some aspects of glyphosate-amphibian interactions need to be better understood and further investigated. At the same time, this body of new research is largely consistent with earlier findings, meaning that MROSD managers can continue current practices regarding glyphosate with more confidence that this will not adversely affect amphibian populations.

Although there is still much uncertainty and controversy surrounding glyphosates and their effect on amphibians, there is currently no compelling reason to recommend prohibiting their use for MROSD land managers, especially in light of the unambiguous benefits of glyphosate in controlling target invasive plants. The best course of action is for land managers to acknowledge the potential vulnerabilities of their local amphibian communities, as well as the general uncertainty surrounding what glyphosate does to amphibians in natural settings. Continually looking for ways to maximize the beneficial effects of glyphosate application while minimizing its exposure to amphibians is the prudent course of action.

Invertebrates

The effect of glyphosate on invertebrates is especially important. Invertebrates play dominant roles in nearly all terrestrial ecosystems. Invertebrates provide crucial ecosystem services such as pollination, herbivory, decomposition, and nutrient cycling. Invertebrates are an essential part of the diet of many organisms. Furthermore, many invertebrates, like insects, are declining globally as has been documented in amphibians, and it is important to understand what role, if any, glyphosate application is playing in this decline. Interestingly, many invertebrates have life histories that parallel those of amphibians; they undergo metamorphosis and may spend egg and larval stages in aquatic environments. Since excess pesticides often collect and concentrate in waterways, this makes these aquatic invertebrates potentially more vulnerable to contaminant exposure, just like amphibians. Six studies were published between 2015-2018 that examined the effects of glyphosate on the health, behavior and food web dynamics of soil and plant dwelling invertebrates (Ferreira-Junior et al. 2017, Baglan et al. 2018, Behrend and Rypstra 2018, Hansen et al. 2018, Niemeyer et al. 2018, Pereira et al. 2018).

Some of the most troubling findings come from work on earthworms. Roundup severely decreased the survival (between 7%-76% surviving after 40 days, compared to 100% surviving in all controls) and cocoon production (50%-70% reduction relative to controls) of four species of earthworms at glyphosate concentrations that encompassed the range of applied concentrations at a nearby vineyard, and these effects increased with increasing concentration of Roundup (Stellin et al. 2018). Ferreira-Junior et al. (2017) studied the impacts of Roundup on survival, growth and emergence of *Chironomus xanthas*, a tropical fly whose larvae live in freshwater. After 10 day of Roundup exposure body length was reduced at the highest exposure level (12.6 mg/L) and all concentrations (0.49, 1.53, 3.69, and 12.6 mg/L) evaluated reduced head size relative to the control. Roundup had no clear dose response effect on emergence, nor overall survival. While the documented effects on larval morphology are concerning and may well have undetected downstream effects, the tropical context and lack of a survival effect make the implications for MROSD managers minimal.

Only one study published between 2015-2108 examined the effect of glyphosate exposure on insect behavior. Baglan et al. (2018) found that the ability of mosquito (*Aedes aegypti*) larvae to habituate to meaningless visual stimuli was impaired by glyphosate concentrations that could realistically be found in the environment and that the higher the glyphosate concentration the larger the impact it had on learning. While it may be tempting to view any negative effect on mosquitos and other disease vectors as a potential benefit of glyphosate application, this result has broader and more troubling implications for glyphosates potential to disrupt key invertebrate behavioral patterns that may affect their survival.

The effects of glyphosate may differ between laboratory trials and more natural experiments because of unforeseen interactions between glyphosate and the environment. These relevant complexities have recently begun to be addressed in studies that investigate the effect of glyphosate on invertebrate species interactions. For example, wolf spider (*Pardosa milvina*) offspring exposed to glyphosate had increased levels of activity and greater capture success of prey and these effects were larger for males compared to females (Behrend and Rypstra 2018). While glyphosate presence did not alter leaf beetle (*Ceratoma arcuta*) densities, it did decrease the density of their predators (Pereira et al. 2018). This finding provides an example of a potential field mechanism that may generate opposite patterns from a laboratory study. If the beetle's response to glyphosate were measured in isolation under controlled conditions, no changes might have been found. Yet, by reducing predator density, glyphosate may ultimately increase beetle density in field conditions. Interestingly, Pereira et al. (2018) also found that the beetles preferred non-glyphosate-treated plants when given a choice, which may negate any potential predator release effect. Collectively these studies demonstrate that glyphosate can impact food predator-prey interactions with potential consequences to food web dynamics.

Niemeyer et al. (2018) showed that the formulations of commercially available glyphosates may have an effect on soil macroinvertebrates. They evaluated the impacts of four different glyphosate-based herbicides (Roundup Original, Trop, Zapp, and Crucial) on avoidance behavior and reproduction of several soil dwelling macroinvertebrates (earthworms, isopods, and collembolans). Only collembolans avoided plants sprayed with the glyphosate-based herbicides, but no reduction in reproduction was observed in collembolans exposed to high pesticide concentrations. Only recommended concentrations of the pesticide Crucial lowered the feeding activity of macroinvertebrates. Though certain formulations had an effect on certain species of soil macroinvertebrate, it is unclear what the long-term survival consequences of these effects are for the invertebrates. Likewise, it is unknown whether the observed effects are due to other chemicals present in the formulations, the interactions of those chemicals with glyhposate, or simply glyphosate itself. Lastly, von Merey et al. (2016) determined that glyphosate applied at recommended rates had no effect on the survival and reproduction of detritivores (earthworms and springtails) or predatory mites.

The current research on glyphosate's effect on invertebrates suggest there is some cause for concern and caution in the application of glyphosate for land management activities. As always, land managers must carefully weigh these potential costs to invertebrates against the known benefits of using glyphosate for invasive weed management. In the only study to explicitly consider these tradeoffs, Hansen et al. (2018) compared the effects of glyphosates to multiple alternative management approaches: hand-pulling, and leaving invasive species untreated. The authors found that hand -pulling and herbicide had the same impact on soil arthropod abundance. More studies that compare the impacts of herbicides to other management options are desperately needed to properly understand the complex interactions between these herbicides, the non-target organisms exposed to them, and the overall ecosystem. Until such studies exist, land managers are forced to extrapolate from laboratory or mesocosm studies with complex findings and questionable relevance to their specific application.

Plants

The purpose of using glyphosate is to remove invasive plant species with the overarching objective of restoring native plant communities that support biodiverse ecosystems. The amount of herbicide that contacts non-target plants is minimal when glyphosate is applied directly onto target plants with hand-held wands and sponges and in the absence of wind and rain. Inevitably though some glyphosate will contact non-target plants. However, damage or death of non-target plants depends on both the plant's characteristics and the amount of herbicide exposure. Only two papers published between 2015-2018 had any relevance to MROSD glyphosate use. Little is known about how glyphosate impacts seeds of non-target plants in the soil seedbank. Turedi et al. (2018) evaluated the toxicity of glyphosate to seeds of three non-target pine species: *Pinus nigra*, *Pinus sylvesteris*, and *Pinus pinaster*. These pines are used in forestry around the world. Although none of the three pines examined in this study are native to California the study is somewhat relevant because the authors demonstrated that seeds of congeners can vary in their sensitivity to glyphosate. In this study, seeds were submerged in Roundup of various concentrations. In general, soaking *Pinus* spp. seeds in glyphosate greatly delayed germination success and the higher the concentration the larger the effect. In actual field practices plant seeds on the surface or buried in soil would not be submerged in herbicide, but could encounter trace amounts. Still, this research shows that seeds of different species in a soil seedbank may be differentially influenced by glyphosate. More research is needed to determine if realistic glyphosate exposure scenarios have impacts on seed viability.

Fortunately, what is perhaps the most relevant (to MROSD) study on glyphosate's effect on non-target plants is also the most optimistic. Stark et al. (2019) found that glyphosate was undetectable in wild growing leafy greens from 3 separate urban sites where low-income community members often forage for wild edibles. Furthermore, the nutritional content of these leafy greens was equivalent to the most nutritious commercially available leafy greens. This result is consistent to our current understanding of glyphosate degradation and should do much to reassure an understandably wary public about the health implications of glyphosate. However, one limitation of this study is that the local applications of glyphosate are not described and might be presumed to be low, since only urbans sites were considered.

There is little new research on glyphosate's effect on non-target plants, and the work that has been done verifies that MROSD's current application methods appear to be both safe and effective. The overall lack of new work, and the limited relevance of new studies to MROSD suggest that MROSD should continue to apply glyphosate judiciously and continually revise procedures and practices in light of new findings.

Soil microbial communities

The effect of glyphosate on soil microbial communities depends on many different factors including the concentration and formulation of glyphosate, soil pH, organic matter content, and exposure time (Nguyen et al. 2016, Nguyen et al. 2018). Soil microbes can be exposed to glyphosate residues if spray is not intercepted by target plants or if rain rinses it of off plants. Because MROSD does not broadcast spray, very little herbicide is likely to be applied directly onto the soil, and because herbicides are not used when rain is forecasted, it is unlikely to be rinsed from plants into the soil.

Four empirical papers published between 2015-2018 studied the effects of glyphosate on microbial communities. When glyphosate was applied at the recommended application it had no significant effects on the abundance, richness, evenness, or community composition of nematodes, bacteria, archaea, or the enzymatic activity of the soil microbes (Allegrini et al. 2015, Dennis et al. 2018, Hernandez Guijarro et al. 2018, Liu et al. 2018). Other studies found that microbial communities did shift following glyphosate exposure (Newman et al. 2016). In another study, fungal abundance declined in the soils exposed to both the recommended and 10-fold higher doses of glyphosate (Liu et al 2018). An increased frequency of a gene that confers glyphosate tolerance was observed in soils after been treated with glyphosate at a 10-fold higher rate than recommended, while the recommended application rate did not increase the prevalence of the glyphosate resistance gene in soil microbial communities (Liu et al 2018). Collectively these studies suggest that a single application of glyphosate at the recommended dose poses little threat to soil biodiversity and function.

Mycorrhizal fungi

Mycorrhizal fungi warrant special discussion due to the intimate and important role they play in plant physiology. Mycorrhizal fungi grow in or on the roots of plants and extend hypha into the soil. These root-like structures acquire soil nutrients and water for their plant hosts and provide protection against pathogens. Invasive plants can disrupt the positive interactions between native plants and their belowground mutualistic mycorrhizal fungi (reviewed in Grove et al. 2017). The management of invasive plants may also impact mycorrhizal mutualisms. Glyphosate in particular may have toxic effects on mycorrhizal fungi because unlike animals, mycorrhizal fungi employ the same biochemical pathway that is targeted by glyphosate in plants. Between 2015 and 2018 eight studies were published that evaluated the impacts of glyphosate exposure on the abundance, diversity, and community composition of mycorrhizal fungi. These studies have reported mixed results, suggesting that the impacts of glyphosate on mycorrhizal fungi are context dependent. In a lab experiment, Roundup did not affect the survival of mycorrhizal fungi associated with an endangered orchid (*Pterostylis arenicola*), but did decrease the rate of hyphal growth in agar (Jusaitis 2018). Findings from this study suggest that the impacts of the invasive fern that invades the orchid’s habitat are more detrimental to the persistence of the orchid than the impact of glyphosate on the mycorrhizal fungi in which the orchid depends upon.

Invasive plants often have less dependence on mycorrhizal fungi than native plants (Pringle et al. 2009) which generally has negative effects on the abundance of mycorrhiza associated with native plants. In the only study of its kind, Maltz et al. (2016) compared the impacts of a non-mycorrhizal plant (*Brassica nigra*) with the effects of removing the plant with glyphosate and found that removing the plant with glyphosate increased the abundance of mycorrhizal fungi that associated with native plants.

The community composition of mycorrhizal fungi changed and the number of viable mycorrhizal spores declined following a single application of a glyphosate-based herbicide (Druille et al. 2015). Similarly, four annual glyphosate applications reduced the abundance of viable spores by 56%, but the proportion of plant roots colonized by these fungi was not influenced by the herbicide, suggesting that the plants were not limited by mycorrhizal propagules (Druille et al. 2016). While Helander et al. (2018) found that spraying glyphosate at 2x the recommend rate decreased the proportion of grass roots colonized by arbuscular mycorrhizal fungi, Nivelle et al. (2018) found that glyphosate had no effect on the abundance or enzymatic activity of arbuscular mycorrhizal fungi. In general, new work shows glyphosate has minimal effects on mycorrhiza, and when effects are observed, there do not appear to have meaningful long-term consequences.

2.1.7 Reptiles

There were four studies published between 2015 and 2018 that evaluated impacts of glyphosate on reptiles. Carpenter et al. (2016) evaluated the effect of two glyphosate-based herbicides on the New Zealand common skink (*Oligosoma polychrome*). Yates Roundup Weedkiler and Agpro Glyphosate 360 were applied to straw at recommended application rates and skinks were kept in boxes with the contaminated straw for four weeks. Exposure to these glyphosate-based herbicides had no effect on skink body weight. However, skinks exposed to Yates Roundup Weedkiller (but not Agpro Glyphosate 360) exhibited heat seeking behaviors, presumably in attempt to increase their body temperatures. The researchers speculate that skinks may have engaged in heat seeking behavior in order to increase metabolic rates to help counteract physiological stress associated with the herbicide. Because only one of the two glyphosate-based herbicides tested had an effect on heat seeking behavior it is plausible that something other than glyphosate in Yates Roundup Weedkiller caused the behavior changes. In another study, Burella et al (2018) found that glyphosate applied to broad-snouted caiman eggshells caused oxidative damage to caiman embryos, but did not elicit an antioxidant defense. The glyphosate exposure did not affect caiman egg viability or hatchling size. Schaumberg et al (2016) found that glyphosate applied to tegu lizard eggs had no effect on egg viability, weight or length at birth or at six months. Of the three tests that were performed to evaluate DNA damage, embryonic glyphosate exposure increased DNA damage in one test, although this response was not dose dependent. Results from the other two genotoxicity test showed no effect of embryonic glyphosate exposure. Of the studies published between 2015 and 2018 the western fence lizard was the only reptile studied that occurs in on MROSD property that was evaluated. Consistent with previous studies, Weir et al (2016) found that glyphosate is not toxic to western fence lizards. Taken together these studies suggest that glyphosate does not affect the survival, growth, or fecundity of reptiles and therefore unlikely to affect population persistence.

2.1.8 Other animals

No studies were published between 2015 and 2018 that addressed the non-target impacts of glyphosate on mammals or birds.

2.1.9 Environmental Fate

Glyphosate concentrations have been detected in agricultural areas in the range of 1.4 to 3.7 mg/L (Giesy et al. 2000, Solomon and Thompson 2003). To my knowledge no studies have reported residual glyphosate concentrations in soils or water in or near to natural areas where glyphosate is conservatively used to control invasive species. However, it seems reasonable to assume that because far less herbicide is applied per area in natural areas compared to agricultural areas, that residual glyphosate concentrations in natural areas are likely to be significantly lower than in agricultural areas.

Here I synthesize 8 studies published between 2015 and 2018 that evaluated factors that contribute to glyphosate mobility and degradation in soil. Glyphosate readily binds tightly (adsorbs) to clay particles in soil and to organic matter. When glyphosate is adhered to clay soil particles it is immobile and cannot interact with non-target organisms. When glyphosate is mobile in soil it is primarily degraded by soil microbes, which covert it first to AMPA or glyoxylic acid, and then ultimately to carbon dioxide (Henderson et al. 2019; NPIC fact sheet). Several studies published between 2015 and2018 have demonstrate that the rate of glyphosate degradation and demobilization in soil is highly variable and depends upon underlying soil properties (Sviridov et al. 2015, Cassigneul et al. 2016, Okada et al. 2016, Nguyen et al. 2018). Two studies concluded that soil acidity strongly inhibits glyphosate degradation (Nguyen et al. 2018). Bento et al. (2016) determined that glyphosate degradation also depends on the water content and temperature of soils. Similarly, Richardson et al. (2018) determined that the amount of glyphosate in runoff was primarily driven by the wetness of the soil at the time of the application and suggest that glyphosate mobilization can be minimized by avoiding spraying when soils are wet. Some work suggests that glypohsate-based herbicides can persist in water from 7 to 70 days, and that the duration of persistence depends on environmental conditions (Giesy et al. 2000). Hernandez Guijarro et al. (2018) compared the rate of glyphosate degradation in soils with and without previous glyphosate exposure and found that repeated exposure to glyphosate impacted neither the microbial community composition nor the rate of glyphosate degradation. This result suggests that initial glyphosate exposure does not appreciably change the microbial soil community nor its ability to metabolize glyphosate.

Collectively the studies published since 2015 that have evaluated glyphosate persistence in soil have provided confirmatory evidence that glyphosate has a high adsorption capacity and is quickly consumed and degraded by soil microbes. Both of these processes minimize the risk that glyphosate will have ecological, biological, or human health consequences beyond its intended purpose. MROSD land managers can continue to judiciously apply glyphosate as an effective and minimally destructive method to control invasive plants.

2.1.10 Emerging concerns

Recent work on the effects of glyphosate on people, ecosystems, and non-target organisms mostly confirms the earlier consensus that glyphosate has minimal environmental consequences when applied correctly. However, some of this work suggests glyphosate might not be as benign as was previously assumed. Results showing a definitive dose-dependent effect of glyphosate are rare, and the gravity of the consequences is often unclear even for clearly demonstrated effects of glyphosate. Perhaps the biggest emerging concern with glyphosate is the emerging picture of contradictory or confusing results. Numerous studies demonstrated a potentially important effect of glyphosate that couldn't be replicated at higher or lower concentrations. While some studies suggest glyphosate has mutagenic or even carcinogenic properties, many other well-designed studies do not. Glyphosate's effect on some non-target organisms is an emerging concern, but here again there is seldom clear or definitive picture of what glyphosate does to organisms and what the ultimate effect is to survival, population growth, and ecosystem function. In summary, the emerging concerns surrounding the unintended effects of glyphosate are complex. Some specific cases that are cause for concern include glyphosate's negative effect on the gut microbiome of honey bees and the potential consequence for susceptibility to pathogens, occasionally reported lethal effects on amphibians, complex results regarding sublethal effects on amphibians, and sometimes serious effects on invertebrates. Again, the biggest concern is the complex and sometimes contradictory story these results tell when considered together. More work must replicate and expand on the few studies that do suggest problematic effects of glyphosate.

2.1.11 Recommendations and Actions

Current actions of MROSD land managers are appropriately cautious and responsible given the continuing uncertainty surrounding glyphosate. Undergirding this uncertainty is the fact that glyphosate is easily the most intensively studied herbicide in this report, and most studies report no meaningful unintended effect of its use. MROSD's application methods for glyphosate are far more conservative than the agricultural applications that account for the vast majority of glyphosate's use. Indeed, much of the recent work is designed to test these higher intensity and less discriminate uses of glyphosate in agricultural settings. This means that the concentration of glyphosate that MROSD introduces into ecosystems is generally well below the levels that cause any effect in these studies. However, the focus on agriculturally relevant concentrations also means that little work has been done that is directly relevant to MROSD’s land management-oriented uses of glyphosate. Given this, I recommend that MROSD continue to use glyphosate sparingly when there is a clear benefit to invasive plant control, and potential negative consequences can be mitigated.

Table 1: Summary of non-target effects of glyphosate published between 2015 and 2018.

Organism	***Findings published from 2015-2018***	***References***
Bees	*In Hawai'i occurance of glyphosate in honey was predominatly in hives near agricultural areas and golf courses. Findings from this study suggest that glyphosate use associtaed with natural areas may not contribute to honey contamination on a large scale.*	*Berg et al. 2018*
	The detected concentrations of glyphosate residues in tree flowers was 1.6 to 8 times lower than the maximum limits allowed according to national and EU standards.	*Cebotari et al. 2018*
	Bee visitation to soybean flowers was not influenced by glyphosate applications. Time following herbicide application did not influence bee visitation. These findings suggest that bees do not avoid flowers contaminated with glyphosate.	*Fagundez et al. 2016*
	Bees preferred sugar water with 10 ppb of glyphosate over the sugar water control, but did not discriminate between the sugar water control and sugar water contaminated with higher glyphosate concentrations. These finding suggest that bees do not discriminate against glyphosate treated food.	*Liao et al (2017)*
	Bee placed in immediate contact with plants wet with herbicide had a ~20% increase in mortality relative to a control group, but bee mortality was not influenced glyphosate if it was allowed to dry prior to contact.	*Abraham et al. 2018*
	Bee larvae fed 0, 0.8, 4 or 20 mg/L of glyphosate. The lowest concentration of glyphosate (0.8mg/L) had no effect on larval mortality, but the two higher concentrations increased mortality. Larval weight decreased for the 0.8 and 4 mg/L treatments. Larval development rate was not affected by glyphosate regardless of the concentration.	*Dai et al. 2018*
	Royal jelly production and the amount consumed by bee larvae was not affected by glyphosate.	*Faita et al. (2018)*
	Bee mortality and ability to detect odor was not influenced by glyphosate.	*Mengoni Goñalons et al. (2018)*
	Bees exposed to glyphosate had reduced abundance of important gut bacteria and were more susceptible to pathogen infection	*Motta et al. (2018)*

Organism	***Findings published from 2015-2018***	***References***
	Bees exposed to 10 mg/L of glyphosate contaminated food had longer flights when traveling a direct path, but that lower doses of glyphosate had no effect on flight time.	*Balbuena et al. 2015*
Butterflies	*There is a negative relationship between monarch abundance and county level glyphosate use on corn and soybean crops suggesting that glyphosate and the abundance of land in the Midwest used for growing glyphosate resistant corn and soybean are contributing factors to population declines of Monarch butterflies*	*Saunders et al (2018)*
Insects	*The learning ability of the mosquito larvae was impaired by glyphosate concentrations that could realistically be found in the environment.*	*Baglan et al. 2018*
	Wolf spider offspring from females exposed to glyphosate had higher levels of activity and greater capture success of prey and these effects were larger for males compared to females.	*Behrend and Rypstra (2018)*
	Body length of Chironomus xanthas*, a tropical freshwater insect, was reduced following 10 days of Roundup exposure at the highest exposure level (12.6 mg/L) and all Roundup concentrations tested resulted in smaller head size. There was no clear effect of the Roundup on emergence.*	*Ferreira-Junior et al. 2017*
	Weeding by hand and the glyphosate-based herbicide, Buccaneer Plus, had the same impact on soil arthropod abundance.	*Hansen et al. (2018)*
	Earthworms, isopods, or collembolas did not avoid oats following one application of glyphosate-based herbicides.	*Niemeyer et al. (2018)*
	Density of a leaf beetle pest was not different in soybean fields that were sprayed once with glyphosate and fields where weeds were hand pulled.	*Pereira et al. 2018*
	Survival and reproduction of detritivores (earthworms & springtails) and predatory mites were not influenced by either glyphosate or AMPA (a biproduct of glyphosate) exposure	*Von Merey et al. 2016*
	Invertebrate richness and community assemblages before and after glyphosate was used to treat invasive willows in New Zealand wetlands were not impacted by glyphosate.	*Wech et al. 2018*
Soil fungi, bacteria & archaea	*Glyphosate had no effect on the abundance, richness, evenness, or community composition of nematodes, soil bacteria or archaeal communities, or the enzymatic activity of the soil microbes. These findings suggest that a single application of glyphosate at the recommended dose poses little threat to soil biodiversity and function. One short coming of this study is that the soils were*	*Dennis et al. 2018*

Organism	***Findings published from 2015-2018***	***References***
	collected from an agricultural area, where glyphosate is routinely used. It is possible that herbicide-intolerant organisms were already depleted.	
	In field of with contrasting textures glyphosate degraded regardless of previous glyphosate exposure. The degradation of glyphosate did not result in changes in microbial communities, likely because many microbes can use glyphosate as a source of nitrogen, phosphorus, and carbon.	*Hernandez et al. 2018*
	Six months after the glyphosate treatments were applied neither the recommended application rate or the 10 -fold higher rate effected bacterial communities. The recommended rate did not increase the abundance of a gene that confers glyphosate tolerance, while the 10-fold higher rate did Fungal abundance declined in the soils exposed to both the recommended and 10-fold higher application rates of glyphosate	*Liu et al. 2018*
	Glyphosate had no effect on the abundance of arbuscular mycorrhizal fungi. The herbicide also had no effect on dehydrogenase or alkaline phosphatase activity which are enzymes produced by microbes such as mycorrhizal fungi to release minerals from unusable forms into usable forms.	*Nivelle et al 2018*
	Roundup reduced the proportion of roots colonized by arbuscular mycorrhizal fungi on forage grass.	*Helander et al. 2018*
	Roundup did not affect the survival of mycorrhizal fungi associated with an endangered orchid, but did decrease the rate of hyphal growth in agar.	*Jusaitis 2018*
	The abundance of viable arbuscular mycorrhizal fungal spores decreased following a single application of the recommended rate of Glacoxan, a glyphosate-base herbicide. Spore viability following the herbicide application varied between AMF species. The community composition of mycorrhizal spores was also influenced by the herbicide. Glyphosate also reduced the abundance of nitrogen fixing bacteria that associate with legume plants.	*Druille et al. 2015*
	Glyphosate used to remove Brasica nigra, an invsive non-mycorhizal plant, increasedt the hyphal abundance of mycorhizal fungi while mowing had no effect on mycorhizal fungal abundance.	*Maltz et al. 2016*

Organism	***Findings published from 2015-2018***	***References***
	Densities of viable arbuscular mycorrhizal fungal spores and free-living diazotrophs decreased following four annual glyphosate applications. However, despite less propagule abundance, mycorrhizal root colonization on Lolium arundinaceum *was not affected by the glyphosate applications. Glyphosate also reduced the proportion* Lolium arundinaceum *roots colonized by dark septate endophytes.*	*Druille et al. 2016*
Amphibians	*Roundup had no effect on tadpole survival or mass for the 3 North American frog species (Blanchard's cricket frog, gray tree frog, and green frog).*	*Boone 2018*
	Glyphosate exposure reduced post metamorphic juvenile frog survival by 37%, changed bacterial community composition on tadpole skin, but did not change peptide excretions. The ability to inhibit chytrid fungus was not influenced by glyphosate exposure. Glyphosate did not affect size, mortality, or immune defense traits of post metamorphic juvenile adult frogs.	*Krynak et al. (2017)*
	The number Rhinella arenarum *cells with damaged DNA increased by 55% following exposure to a glyphosate-based herbicide.*	*Soloneski et al. 2016*
	African clawed frog tadpole mortality was not influenced by Roundup, but the tadpoles exposed to 4.5 and 9 mg/L were smaller than the controls	*Wagner et al. 2017*
	Glyphosate had no effect on either development or size of the neotropical frog Physalaemus cuvieri, but did increase nose to snout and eye width asymmetry	*Costa and Nomura 2015*
	Glycogen was found in lower concentrations in bullfrogs exposed to Roundup, suggesting the frogs may have to expend additional energy to maintain homeostasis. Total lipids were also found in lower concentrations in bullfrogs exposed to Roundup. This may be because the lipids are being mobilized as a stress response. The biochemical changes due to glyphosate exposure suggest the frogs were experiencing stress. Bullfrog growth and mortality was not affected by Roundup.	*Dornelles and Oliveira 2016*
	Glyphosate treatments did not affect tadpole mortality, development, and body length to width ratio. However, when the tadpoles were reared at 15° C their tail lengths were slightly shorter when exposed to Roundup.	*Baier et al. 2016*
	Skin thickness increased with exposure to glyphosate and Roundup original, but Transob did not influence skin thickness. Glyphosate reduced tadpole metabolic rate during hypoxia, while Transorb increased metabolic rate, and Roundup did not affect metabolic rate.	*Rissoli et al. 2016*

Organism	***Findings published from 2015-2018***	***References***
	Glyphosate had no effect on erythrocytic change in Dendropssophus minutus blood cells	*Carvalho et al. 2018*
	Glyphosate treatments had mixed effects on corticosterone production, at 1 mg/L glyphosate increased production by 91%, whereas 2 mg/L of glyphosate had no significant effect. Both 1 and 2 mg/L glyphosate treatments increased tadpole metabolic rate. Altered corticosterone levels and increased metabolic rate can result in increased energy expenditure which could impact tadpole survival and growth.	*Burraco and Gomez-Mestre 2016*
	Tadpoles exposed to glyphosate continuously for 36-61 days produced increased concentrations of a chemical defense compound.	*Bokony et al. 2017*
	Wood frog tadpoles do not reduce movement when exposed to both chemical predator cues and Roundup. Tadpoles should reduced activity when they perceive risk of predation.	*Moore et al. 2015*
	Exposure to Glyphogan Classic, a glyphosate-based herbicide widely used in Europe did not influence the survival, behavior, body mass and predatory activity of smooth newts.	*Ujszegi et al. 2015*
	Glyphosate caused 100% mortality of agile frog tadpoles in the lab, but had no effect on mortality in the mesocosm. The outcomes of herbicide impact studies done in a lab and mesocosms could be different for a number of reasons including different rates of chemical degradation, increased ecological complexity, herbicide stratifying in water due to temperature variation. To date much of understanding regarding pesticide impacts on amphibians come from lab experiments	*Miko et al. 2015*
	Spotted salamander tadpole survival was not affected by glyphosate in either the low or high light conditions. The proportion of tadpoles that metamorphosed increased with herbicide exposure under both light regimes, suggesting that the herbicide was a stressor to the tadpoles. At moderate light levels glyphosate decreased the immune response of the tadpole, but at low light glyphosate did not change the immune response. Glyphosate may have larger impacts on spotted salamander tadpoles in low light environments than out in the open.	*Levis and Johnson 2015*
Birds	*No new information*	
Lizards	*Consistent with previous studies, glyphosate is not toxic to western fence lizards.*	*Weir et al. 2016*
	The body weight of skinks was not impacted by being sprayed with commercial glyphosate formulations. However, one of the glyphosate formulations, Yates Roundup Weed killer, increased skink body temperatures for 3 weeks following	*Carpenter et al. 2016*

Organism	***Findings published from 2015-2018***	***References***
	exposure. Increased body temperatures may be a response to increase metabolism and thereby counteract physiological stress.	
	Glyphosate applied to tegu lizard eggs had no effect on egg viability, weight or length at birth or at six months. Of the three test that were performed to evaluate DNA damage, embryonic glyphosate exposure increased DNA damage in one test, although this response was not dose dependent. Results from the other two genotoxicity test showed no effect of embryonic glyphosate exposure.	*Schaumburg et al. 2016*
	Glyphosate applied to broad-snouted caiman eggshells caused oxidative damage but did not elicit an antioxidant defense. The glyphosate exposure did not affect caiman egg viability or hatchling size.	*Burella et al. 2018*
Fish	*Environmental relevant concentrations of glyphosate reduced sperm quality of yellowtail tetra fish. Viability of sperm cells was impaired at 300 µg/L, and sperm motility was impaired at 50 µg/L, concentrations that are within legal limits in U.S.A. waterbodies.*	*Goncalves et al. 2018*
	Exposure of zebrafish embryos to high concentrations of Roundup® (50 mg/L) resulted in morphological abnormalities including the head, eye, and brain. Gene expression was altered in two of three brain regions.	*Roy et al., 2016*
	10 mg/L glyphosate exposure negatively altered the morphology, biomechanics, behavior, gene expression, and physiology of larval and embryonic zebrafish.	*Zhang et al. 2017*
	Chronic exposure of gold fish (Carassius auratus) to glyphosate (34 mg/L) disturbed the metabolism in various tissues, led to oxidative stress and renal injury	*Li et al. 2017*
Plants	*Pinus nigra, Pinus sylvesteris, and Pinus pinaster seeds vary in their sensitivity to glyphosate. This research suggest seeds of different species in a soil seed bank may be differentially influenced by glyphosate.*	*Turedi et al. 2018*
	Glyphosate was below the detection limits in naturalized edible leafy greens in neighborhoods in the East (San Francisco) bay. Plant tissue and the nutritional content was equivalent to the most nutritious commercially available leafy greens	*Stark et al 2019*
	Glyphosate and its breakdown product AMPA inhibit antioxidant enzyme activities, causing the accumulation of reactive oxygen species (ROS) that induce physiological dysfunction and cell damage. Both glyphosate and AMPA decrease photosynthesis, but through different mechanisms: glyphosate increases chlorophyll degradation, while AMPA disturbs chlorophyll biosynthesis.	*Gomes et al. 2016*

Table 2: Glyphosate LC50 values reported between 2015-2018.

Formulation	***Organism***	***Common name***	***Hours***	***LC50 (mg/L)***	***Reference***
Credit	*Rhinella arenarum*	*NA*	*96*	*78.18*	*Soloneski et al. 2016*
Aquamaster	*Anaxyrus boreas*	*Western toad*	*24*	*8279*	*Vincent and Davidson 2015*
Aquamaster + Agri-dex	*Anaxyrus boreas*	*Western toad*	*24*	*5092*	*Vincent and Davidson 2015*
Aquamaster + Competitor	*Anaxyrus boreas*	*Western toad*	*24*	*853*	*Vincent and Davidson 2015*
Roundup Original	*Physalaemus cuvieri*	*neotropical frog*	*96*	*2.3*	*Costa and Nomura 2015*
Credit	*Cnesterodon decemmaculatues*	*tenspotted livebearer*	*96*	*86.8-98*	*Ruis de Arcute et al. 2018*
Glifoglex	*Cnesterodon decemmaculatus*	*tenspotted livebearer*	*96*	*41.4-53*	*Brodeur et al. 2016*
Roundup Original	*Chironomus xanthas*	*Fresh water insect*	*48*	*251.5*	*Ferreira-Junior et al. 2017*

Eskoba	*Daphnia magna*	*Cladoceran*	*48*	*29.48*	*Reno et al. 2018*
Roundup Ultramax	*Daphnia magna*	*Cladoceran*	*48*	*11.68*	*Reno et al. 2018*
Panzer Gold	*Daphnia magna*	*Cladoceran*	*48*	*2.12*	*Reno et al. 2018*
Sulfosata Touchdown	*Daphnia magna*	*Cladoceran*	*48*	*1.62*	*Reno et al. 2018*
Eskoba	*Ceriodaphnia dubia*	*Cladoceran*	*48*	*14.49*	*Reno et al. 201*
Roundup Ultramax	*Ceriodaphnia dubia*	*Cladoceran*	*48*	*4.84*	*Reno et al. 201*
Panzer Gold	*Ceriodaphnia dubia*	*Cladoceran*	*48*	*0.54*	*Reno et al. 201*
Sulfosata Touchdown	*Ceriodaphnia dubia*	*Cladoceran*	*48*	*0.31*	*Reno et al. 201*
Glyphosate	*Sceloporus occidentalis*	*Western fence lizard*	*96*	*>1750 μg/g* *(LD50)*	*Weir et al. 2016*

2.2 *IMAZAPYR*

Imazapyr is a non-selective herbicide that can be used to control terrestrial and aquatic vegetation pre or post emergence and is often used to control plants when glyphosate is ineffective. At MROSD imazapyr is used in forest understories to control the invasive vines, periwinkle (*Vinca major*), and English Ivy (*Hedera helix*). Unlike glyphosate, imazapyr is active in the soil and can be taken up by plant roots and should not be applied when non-target plants are interspersed with target plants .

2.2.1 Human health risks

Oral, dermal or inhalation exposure to imazapyr has low toxicity to humans. In 2006, it was determined by the EPA that there is no evidence of carcinogenicity, neurotoxicity, immunotoxicity, or developmental toxicity. There were no studies published between 2015 and 2018 that further evaluated human health risks associated with imazapyr exposure.

2.2.2 Ecological risks

Imazapyr has low acute and chronic toxicity to fish, aquatic invertebrates, birds, and mammals. Only 3 studies were published between 2015 and 2018 that investigated the effects of imazapyr on non-target organisms. One of these 3 studies determined the lethal doses of imazapyr to juvenile frogs, another study evaluated sub-lethal effects on catfish, and the third study examined how non-target aquatic plants were impacted by an imazapyr application.

Babalola et al. (2018) determined that the lethal toxicity of imazapyr on African clawed frog (*Xenopus laevis*) embryos, premetamorphic larvae, and prometamorphic larvae are 36, 32.8, and 173.5 mg/L, respectively. The concentrations that resulted in mortality far exceed concentrations that amphibians encounter in any real world scenarios. Findings from this study confirm that environmentally relevant concentrarions of imazapyr will not have acute lethal effects on frog embryos or tadpoles.

Golombieski et al. (2016) evaluated the effects of an herbicide formulation that contains both imazapyr and imazapic on silver catfish following 96 hours of exposure. Findings from this study suggest that the herbicide formulation puts some stress on fish, but that overall fish health is minimally impacted by 96-hours of glyphosate exposure. This study provides only limited insights into imazapyr toxicity because it is not possible to disentangle the effects of imazapyr and imazapic.

Enloe and Netherland (2017) compared the effects of clethodim, a grass specific herbicide, with two non-selective herbicides, glyphosate and imazapyr, on a target invasive grass and co-oocuring non-target aquatic forbs. Imazapyr and glyphosate reduced the biomass of non-target species by 64-100%, whereas clethodim did not affect native forbs at all. Clethodim, glyphoste and imazapyr all reduced the target grass cover by 69-85%. This study provides further supoort that imazapyr is a non-selective herbicide and that non-target co-occuring plants could be impacted by its use.

2.2.3 Environmental fate

Six studies published between 2015 and 2018 evaluated the movement and persistence of imazapyr in the environment (Porfiri et al. 2015, Douglass et al. 2016, Louch et al. 2017, Ozcan et al. 2017). Imazapyr is soluble in water, adsorbs poorly to soil, and is therefore mobile in the soil with the potential to be leached into ground water or transported to surface waters. In water imazapyr degrades through photolysis (i.e. sun light breaks it down). Porfiri et al. (2015) confirmed that imazapyr weakly adsorbs to soil, and that there is a positve relationship between clay content in soil and imazapyr adsorbtion. Despite imazapyr's mobility potential, two recent studies report non-detectable levels in soil and water in sites where imazapyr had recently been applied (Louch et al. 2017, Ozcan et al. 2017). Douglass et al. (2016) evaluated imazapyr residues in soil under and near to aerial sprayed Tamarisk stands. They determined that 71% of the imazapyr was intercepted by the target plant, which means that 29% was deposited into the surrounding environment. They further determined that there was less imazapyr in the soil under the Tamarisk canopy than outside the canopy, but that degradation rates were 4 times faster outside the canopy. Outside the canopy >99% of the imazapyr degraded within 6 months, whereas under the canopy it took 15 months to degrade 99% of the imazapyr. Collectively these studies suggest that imazapyr degrades quickly in the environment.

Two studies published since 2015 evaluated mechanisms that influence the rate of imazapy degredation in soil and water. Atitar et al. (2018) evaluated the role of pH and light in the degration of imazapyr in water. Thet found that the maximum amount of imazapyr is adsorbed at a pH of 3 and that as pH increases imazapyr adsorption decreases. They further found that the rate of imazapyr adsorption is higher in the light than in the dark, but that the effect that light has on adsorbtion is patially dependent upon pH. Bundt et al. (2015) compared the degradation rates of imazapyr in soils with and without previous exposure to herbicide formulations that contained imazapyr. Previous exposure to imazapyr did not increase the rate of microbial degradation of imazapyr following subsequent applications.

2.2.4 Emerging concerns

Far fewer studies have evaluated ecological and human health risks associated with imazapyr use than with glyphosate use. This discrepancy is likely because of the increased use of glyphosate since the advent of glyphosate resistant crops. Along with herbicide efficacy, this knowledge gap should be considered when choosing which non-selective herbicide to use on target invaders.

2.2.5 Recommendations and actions

To minimize non-target effects on native plants, I recommend only applying imazapyr to target populations that form dense monocultures.

2.2.6 Best management practice (bmp) updates

No updates on BMPs at this time

2.3 AMIOPYRALID

Aminopyralid is primarily used to control invasive species in the Asteraceae, Fabaceae and Apiaceae. At MROSD it has been used to control *Carduus, Cirsium*, *Centaurea*, *Dittrichia*, *Silybum*, *Lathyrus*, and *Delairea species*. Despite being registered for use since 2005, very few studies have evaluated human and ecological risk associated with aminopyralid. The paucity of studies is surprising considering that it is one of the most widely used herbicides worldwide (Randall 1996). Nearly all of the aminopyralid literature published between 2015 and 2018 evaluated its effectiveness in controlling invasive species in agricultural, rangeland, and natural areas (see Annotated Bibliography in Appendix 1 for descriptions of the efficacy studies).

2.3.1 Human health risks

According to the EPA, as of 2005, aminopyralid has low toxicity if eaten, inhaled, or though dermal contact, but it can be an eye irritant. Only one aminopyralid study was published between 2015 and 2018 that relates to human health. Researchers at Dow Chemical, the manufacturers of Milestone, evaluated a method to test for skin sensitivity and trademarked the method as KeratinoSens^{TM}. They found that this new method confirmed that aminopyralid does not irritate skin (Settivari et al. 2015).

2.3.2 Ecological risks

Aminopyralid has low acute and chronic toxicity to fish, aquatic invertebrates, birds, and mammals. No studies were published between 2015-2018 that further evaluated toxicity of aminopyralid exposure on animals. One study was published that examined the impact of aminopyralid on soil microbial communities. Due to concern that cooler temperatures at northern latitudes would slow the degradation of aminopyralid in soil Tomco et al. (2016) compared soil microbial communities in sprayed and unsprayed soils and found no differences in microbial diversity or community composition.

***2.3.3* Environmental fate**

Only two studies published between 2015 and 2018 documented the movement and persistence of aminopyralid in the environment. To determine if aminopyralid degradation occurs slower in cool temperature environments Tomco et al. (2016) sprayed plowed agricultural fields with aminopyralid at the recommended rate. Aminopyralid degraded rapidly over the 29-day period following the herbicide application. After 90 days, residual aminopyralid persisted at a rate of 0.049 μg/g at one site and 0.12 μg/g at the other.

To determine if aminopyralid and other pesiticides were moving from crop fields into bee hives Karise et al. (2017) collected honey from hives that were in the general vicinity of oil seed crops. The honey was analyzed for 47 pesicide residues, includiing glyphosate, aminopyralid, and clopyralid. Aminopyralid was not detected in the honey.

These findings are congruous with the state of knowledge regarding the persistence of aminopyralid in the environment. That is, Tomco et al. (2016) and Karise et al. (2017) provide further support that aminopyralid is likely to be non-persistent and relatively immobile in the environment.

2.3.4 Emerging concerns

No emerging concerns at this time

2.3.5 Recommendations and actions

No additional recommendations or actions at this time.

2.3.6 Best management practice (BMP) updates

No updates on BMPs at this time

2.4 *TRICLOPYR*

Triclopyr is a selective herbicide used to treat woody and herbaceous plants and has little or no impact on grasses or conifers. At MROSD it is used to treat woody shrubs & trees (e.g. *Acacia, Baccharis, Cytisus, Eucalyptus, Genista, Ilex, Spartium)*. It works by mimicking the auxin hormone and causes exposed plants to grow excessively fast and die over the course of several weeks.

2.4.1 Human health risks

Triclopyr has low toxicity if inhaled or eaten, and that it is not well absorbed through the skin. Triclopyr leaves the body through urine with 93-94% excreted within 24 hours (Strid et al. 2018). The salt in triclopyr can cause permanent eye damage. As of 2018, the EPA has not been able to classify the carcinogenicity of triclopyr due to a lack of scientific studies. The EPA has also not tested triclopyr for endocrine disruption potential. Guilherme et al. (2015) evaluated the genotoxic effects of Garlon and its active ingrediant triclopyr on European eels (*Anguilla anguilla*). The eels were expose for either 1 or 3 days to Garlon, or triclopyr, at environmentally releveant concentrations. DNA damage occurred with both exposure durations and with both chemicals. Slightly more DNA damage was observed in eels exposed to Garlon than triclpyr alone. This study, though on fish, is relevent to human helath because DNA damage can trigger carcinogenic processes. To date very little is known about cancer risks associated with triclopyr.

No studies were published between 2015-2018 that evaluated exposure risks to occupational triclopr users. However, in a small pilot study, Gilden et al. (2016) evaluated pesticide exposure in children that play in an atheltic field that was sprayed with a herbicide formulation that contains several active chemicals, inlcuding triclopyr. After playing on the field kids provided urine and shoe wipe samples that were analysed for pesticide residues. All chemicals, including triclopyr, were below the detection levels.

2.4.2 Ecological risks

Six studies were published between 2015 and 2018 that examined triclopyr exposure risks of non-target organisms. Two studies found that triclopyr exposure did not affect red legged or northern leopard frog tadpole mortality (Curtis and Bidart 2017, Yahnke et al. 2017). However, leopard frog growth was inhibited by triclopyr (Curtis and Bidart 2017). Red legged frog behavior and body condition were not alterred following 96 hours of triclopyr exposure, although tadpoles did exhibit lethargy during the exposure period, and had a 1-day delay in completing metamorphose.

Two studies evaluated the effects of triclopyr on fish health. As described above, Guilherme et al. (2015) evaluated the genotoxic effects of Garlon and its active ingredient triclopyr on the European eel (*Anguilla anguilla*) and found that both Garlon and triclopyr caused DNA damage. Slightly more DNA damage was observed in fish exposed to Garlon than triclopyr alone. This study is importatn because DNA damage can be a precursor to seroius health problems that could have population level consequences. Suvarchala and Philip (2016) evaluated the effects of a metabolite of triclopyr on zebrafish (*Danio rerio*) embryos. Zebrafish were exposed to five concentrations of the metabolite (200, 400, 600, 800, and 1000 µg/L). Fish embryos had increased mortality with concentrations > 400 µg/L, a delay in hatching time, and a decrease in percentage of hatched embryos. Triclopyr metabolite exposure also reduced heartbeat rate, blood flow, body and eye pigmentation, and increased pericardial and yolk sac edema. Taken together these studies are congruous with previous work that suggests that triclopyr exposure does not cause acute mortality, but does result in non-lethal health impacts. Further research is need to determine if these non-lethal impacts influence population viability.

The impacts of triclopyr on soil microbial communities were described in two studies that were published between 2015 and 2028. Both studies found that triclopyr altered the community composition of bacterial communities (Souza-Alonso et al. 2015, Marileo et al. 2016). However, in one study the recommended appplication rate of glyphosate had no impact on bacterial communtiy composition, whereas twice the recommended dose did (Marileo et al 2018). In the second study, bacterial communities were only measured following two applications of triclopyr (Souza-Alonso et al. 2015). Fungal diversity and community compositon were not impacted by triclopyr (Souza-Alonso et al. 2015). These studies taken together suggest that bacterial communitues can be impacted by either two applications at the recommended rate or by one application at twice the recomened rate, but that one application at the recommended rate does not effect bacterial communities. Further research is needed to determine if changes in the bacterial community result in changes to ecosystem dynamics (e.g decomposition rates, carbon storrage, nutrient cycling, etc.).

2.4.3 Environmental fate and transport

Only two studies published between 2015 and 2018 contribute to our understanding of the persistence and fate of triclopyr in the environment. Li et al. (2018b) used a new method to evaluate triclopyr residues in forage grass and soil. In forage grass, triclopyr degraded rapidly over the first 14-day period following application. In soil, triclopyr degraded at a relatively slow rate, and dissipated steadily to below detection levels by 60-days post application. The residue concentrations in the forage grass 7 days following the application was lower than maximum limits allowed by the EPA (USA). In a second study, Tayeb et al. (2017) evaluated triclopyr pollution in runoff from a palm plantation in Malaysia. Triclopyr was sprayed onto 0.5 ha of the plantation and runoff samples were collected for 120 days following the treatment. Only 0.025% of the amount of the triclopyr applied was recovered in runoff. These findings suggest that triclopyr in runoff is minimal due to of its short persistence and strong soil adsorption. Both of these studies are consistent with previous research that suggest the triclopyr breaks down relatively quickly in soil, water, and plant tissues (Strid et al. 2018).

2.4.4 Emerging concerns

The finding that DNA damage occurred in eels following expoure to triclpoyr is somewhat concerning. This study, though on fish, is relevent to human helath because DNA damage can trigger carcinogenic processes. To date very little is known about cancer risks associated with triclopyr and further research is warrented and necessary.

2.4.5 Recommendations and actions

Given the paucity of information regarding human health risks associated with occupational use of triclopy and the finding that it damaged DNA in eels it seems that precautions should be used to minimize exposure when handling or applying triclopyr.

2.4.6 Best management practice (bmp) updates

No updates on BMPs at this time

2.5 *CLOPYRALID*

Like aminopyralid and triclopyr, clopyralid is an auxin-mimicking selective herbicide used to control plants in the Asteracea, Fabaceae, Solanacea, and Polygonaceae. Clopyralid is used at MROSD to control *Carduus*, *Cirsium*, *Centaurea*, *Dittrichia*, *Silybum*, *Lathyrus*, and *Delairea odorata.*

2.5.1 Human health risks

Clopyralid has low toxicity if eaten, inhaled, or contacted via dermal exposure. Clopyralid can cause severe and permeant eye damage. If ingested clopyralid is excreted in urine within 48 hours. Though important data gaps occur, there is no evidence that suggests clopyralid is carcinogenic or damaging to the endocrine, immune, or neurological systems. No empirical studies were published between 2015-2018 that evaluated human exposure or health risks associated with clopyralid.

2.5.2 Ecological risks

Only three studies published between 2015 and 2018 evaluated acute or chronic toxicity of clopyralid on non-target organisms. Consistent with previous toxicity studies, Weir et al. (2016) found that environmentally relevant concentrations of clopyralid are not toxic to western fence lizards *(Sceloporus occidentalis*). Two studies evaluated the effects of clopyralid on soil microbial communities and in both studies clopyralid had no effect on the community composition of soil bacterial communities (Marileo et al. 2016, Tomco et al. 2016)

2.5.3 Environmental fate

Clopyralid is a mobile herbicide that weakly adsorbs to soil and is soluble in water. Palma et al (2015) found that the mobility of clopyralid was influenced by soil pH and that the concentrations of clopyralid decreased with increasing soil pH. In contrast, the European Food Safety Authority concluded that the adsorption of clopyralid was not pH dependant (Arena et al. 2018). Clopyralid is degraded by microorganisms in the soil with a half-life that ranges from 15-280 days.

2.5.4 Emerging concerns

No emerging concerns at this time

2.5.5 Recommendations and actions

No additional recommendations or actions at this time.

2.5.6 Best management practice (BMP) updates

No updates on BMPs at this time

2.6 *CLETHODIM*

Clethodim is a selective herbicide used to control post emergent annual and perennial grasses. At MROSD clethodim is used to control slender false brome (*Brachypodium sylvaticum*) and Harding grass (*Phalaris aquatica*).

2.6.1 Human health risks

Clethodim is moderately toxic by ingestion and is classified as practically non-toxic by inhalation or dermal absorption. No studies were published between 2015 and 2018 that evaluated human exposure or health risks associated with clethodim.

2.6.2 Ecological risks

Two studies were published between 2015 and 2018 that contribute to our understanding of risks associated with clethodim exposure to non-target organisms.

Lincoln et al. (2018) evaluated how clothdim applied at different times of year, and at different frequecies, to control invasive grasses in Pacific Northwest praries effects *Camas quamash*, a locally important forb. The herbicide treatment reduced leaf length and increased flower and seed production, but did not influence seed viability or palatability to herbivores. The observed effects suggest that clethodim is not detrimental to *Camas* and that repeated applications can be safely used in areas with high concentrations of *Camas*. Although *Camas* does not occur on MROSD land, other non-grass monocots do. This study provides preliminary evidence that clethodim may be safe to use around non-grass monocots. Further research is needed to determine if other (non-*Camas*) monocots are sensitive to clethodim.

Schultz et al. (2016) investigated the non-target effects of clethodim on *Euphydryas colon* caterpillars. Numerous species in the *Euphydryas* genus are in decline worldwide. The effect of clethodim on caterpillar survival was inconclusive. However, clethodim exposure doubled the concentration of the aucubin in the caterpillars. Aucubin is a defense compound in the caterpillar host plant that is sequestered by the caterpillars. The increase found in the caterpillars likely reflects an increase in the concentration in the host plants in response to the herbicide. It’s possible that changes in the abundance of defense compounds in the caterpillars may decrease predation.

2.6.3 Environmental fate

Two studies were published between 2015 and 2018 that inform our understanding of clethodim persistence in the environment. In a controlled lab study, Sandin-Espana et al. (2016) evaluated the rate of photolytic degradation of clethodim and determined that sunlight has a more prominent role in the breakdown of clethodim than was previously thought. Jose Villaverde et al. (2018) further investigated the process of photo degradation of clethodim in water and determined that it breaks down quickly (within hours) in water, but that the breakdown products may be more toxic to bacteria than clethodim itself. They further concluded that the degradation products of clethodim have a greater potential to be leached than clethodim.

2.6.4 Emerging concerns

No emerging concerns at this time

2.6.5 Recommendations and actions

If/when applying clethodim near to host plants of special status butterflies consider spraying either before caterpillar larvae are present or after they pupate.

2.6.6 best management practice (BMP) updates

No updates on BMPs at this time

3.0 ADJUVENTS/SURFACTANTS: *Alcohol ethoxylate, Alkylphenol ethoxylate, Lecithin, and Canola oil: ethyl & methyl esters*

Only 2 studies were published between 2015 and 2018 that evaluated the effects of surfactants used by MROSD on non-target organisms. To my knowledge no studies were published during this time frame regarding the use of MROSD surfactants on human health or on their persistence in the environment.

3.1 Ecological Risks

Eivazi et al. (2018) evaluated the effect of a surfactant blend that contained alkylphenol ethoxylate and alcohol ethoxylate on microbial enzymes involved in nutrient cycling in two soils that differed in texture (i.e. clay content). The surfactant was applied to the soils at the recommended application rate and double the recommended application rate. The surfactant treatments had an inhibitory effect on enzymatic activity compared to the control in both soils, but also increased microbial biomass. One challenge that arises from interpreting these findings is that the decomposition of the treated plants is likely confounded with the effects of the chemicals. Plant decomposition can increase microbial biomass by providing detritivores with increased organic nutrients.

Wang et al. (2015) evaluated the effects of anionic, cationic, and non-ionic fatty alcohol polyoxyethylene surfactants on the larval behavior of zebrafish. To the best of my knowledge only the fatty alcohol polyoxyethylene surfactant treatment is relevant to MROSD**.** The fatty alcohol polyoxyethylene surfactant decreased larval zebrafish activity, but had no effect on body size relative to the control group.

4.0 FUNGICIDE

4.1 *PHOSPHITE K*

No studies were published between 2015 and 2018 that describe human health risks, ecological effects, or the environmental fate of the fungicide Phosphite K.

5.0 INSECTICIDES

5.1 *DIATOMACEOUS EARTH*

No studies were published between 2015 and 2018 that describe human health risks, ecological effects, or the environmental fate of the diatomaceous earth used as an insecticide.

5.2 *D-TRANS ALLETHIRIN*

No studies were published between 2015 and 2018 that describe human health risks, ecological effects, or the environmental fate of the D-trans allethrin used as an insecticide.

5.3 *FIPRONIL*

Fipronil is a broad-spectrum phenylpyrazole insecticide that blocks gamma-amino butyric acid (GABA) receptors in the central nervous system of insects, leading to hyper-excitation and death. At MROSD fipronil is the active ingredient in Maxforce bait stations to control Argentine ants. MROSD only uses fipronil indoors, so exposure risks to non-target organisms is negligible.

5.3.1 Human health risks

Fipronil is moderately toxic if ingested or inhaled. Toxicity from dermal contact is low. The EPA has classified fipronil as a possible human carcinogen. In 2004, Chodorowski and Anand found that rats exposed to fipronil produced excessive thyroid cells that lead to tumor formation. No studies were published between 2015 and 2018 that further evaluated links between fipronil exposure and cancer. There were four studies published between 2015 and 2018 that evaluated exposure and human health risks associated with fipronil.

Aerts et al. (2018) evaluated pesticide residues on vinyl wrist bands worn by people associated with an Environmental Studies program at a University in Belgium. None of the people in this study used fipronil occupationally and no information was collected regarding exposure to indoor bait stations. Results from this study suggest that fipronil exposure is highly individualized. Fipronil was detected on wrist bands worn by 10 out of 30 participants in concentrations that ranged from 4.8-90 ng/g. The participants in this study also filled out a survey that described their diets and demographics. Four of the participants that had been exposed fipronil had pets that used a pet care product that contained fipronil. Smoking and vegetable rich diets were correlated with consumption with higher cumulative concentrations of pesticide residues in wristbands.

Lu et al. (2015) evaluated the effects of fipronil and its metabolite, fipronil sulfone, on important endocrine functions. They found that fipronil and fipronil sulfone did not influence estrogenic processes. However, fipronil sulfone had a negative effect on thyroid hormone activity. Mitchell et al. (2017) investigated the effect of fipronil exposure on molecular pathways in the liver, specifically they evaluated changes in non-coding RNA. Non-coding RNA does not result in protein synthesis, but does perform other essential functions. When liver cells were exposed to 10 mM of fipronil 76 genes were upregulated and 193 were down regulated, that is out of 56,384 genes 269 behaved differently following fipronil exposure. It is unknown what, if any, effects these changes have on human health, these changes in gene expression could lead to changes in cellular function.

Ruangjaroon et al. (2017) evaluated the effects of fipronil on cellular processes that are thought to be important in the development of neurodegenerative diseases. Fipronil induced structural changes in SH-SY5Y cells and shortened neurite outgrowth projections. These changes could lead to neurodegenerative diseases (e.g. Alzheimer’s, Parkinson’s, etc.). They conclude that fipronil is a risk factor for neuronal degeneration, which in the long-term could result in neuronal diseases.

All of the studies published between 2015 and 2018 provide further evidence that fipronil is toxic to humans and can interfere and damage numerous systems in the human body (e.g. neurological, endocrine, and immune systems). These studies contribute to the growing body of evidence that fipronil may have negative effects on human health.

5.3.2 Ecological risks

There were X studies published between 2015 and 2018 that evaluated fipronil impacts on non-target organisms including 4 studies on amphibians, 18 studies regarding bees, and 2 studies on birds.

Four studies examined factors that influence exposure risks of amphibians to fipronil. These studies do not describe the effects of exposure to amphibian health or population viability. Glinski et al. (2018) determined that the concentration of fipronil in toad liver tissues decreased to nearly 0 within 24 hours following exposure to soil sprayed with the maximum recommended application rate. However, the fipronil metabolite, fipronil sulfone, increased over this time frame. This study does not attempt to place these results in context of toad health, making it hard to interpret the significance of these rates. Gripp et al. (2017) evaluated oxidative stress in tadpoles following exposure to soil that was treated with 35, 120 or 180 μg/kg of fipronil. Though the concentrations used in this study were high, they were not lethal to tadpoles following 7 days of exposure. Most of the antioxidant enzymes evaluated in this study were not altered by fipronil (or its metabolites) in a dose dependent way. Van Meter et al. (2015) evaluated the effects of fipronil that contacted green tree and barking frogs through either spray or by through contact with treated soil. Unsurprisingly, eight hours following fipronil application treatments, both species of frogs that were sprayed with fipronil had higher It is unclear what the consequence of these concentrations of fipronil in liver tissue means for frog health, but does suggests that when fipronil is in contact will soil it becomes somewhat immobilized and poses less risk to amphibians . In a separate study, Van Meter et al. (2016) applied fipronil to soil with high and low organic matter content then put toads in contact with the treated soils for 8 hours. Fipronil concentrations in toad tissues were greater when the toads had been on the low-organic matter soil than the high-organic matter soil. Collectively these studies provide further support that environmental factors will influence the extent that amphibians are exposed to fipronil.

Bees

In the 1990s there was a large die off of honey bees and neonicotinoids were thought to be responsible. Holder et al (2018) make a strong argument that the bee die off was more likely due to fipronil. Fipronil is used as a seed coat on sun flower crops (and other crop seeds) and translocates to all plant tissues including flowers. Sun flower plants are in flower for several weeks providing bees constant exposure to fipronil during that time. Holder et al (2018) used a multi-pronged approach to investigate the likelihood of fipronil as a driving agent in bee mortality. They quantified the toxicity of fipronil and neonicotinoids, and then incorporated the mortality rates into a demographic model. They also fed bees non-lethal doses of fipronil and found that almost all the ingested fipronil was present 6 days later, suggesting that it can bioaccumulate with continued exposure. Other research that fipronil contamination is not present in honey bee hives. For example, Antunez et al. (2015) determined that fipronil was not stored in pollen in any colony or season. This study is in contrast to similar studies and suggests that the risk of fipronil contamination varies between locations. Vespex is a pesticide that contains fipronil to control wasps. To determine of fipronil is entering honeybee hives Edwards et al (2018) measured these chemicals in worker bees, bee larva, honey and pollen. Of the 320 samples comprising honey, worker bees, bee larvae and pollen that were examined, only one worker bee from the wasp treatment area contained fipronil residue. The authors conclude that the use of Vespex traps to control wasps poses little to no risk of entering bee hives and should support bee populations by reducing wasp predation. Collectively, these studies provide evidence that fipronil contamination can sometimes be an issue for honeybees while other evidence suggests that other times fipronil exposure does not occur. As is always the case, the risk of pesticide exposure depends upon the concentrations deployed into the environment. The use of wasp traps that contain fipronil appear to be safe for honey bees, while coating seeds in fipronil appears to pose substantial exposure risk to honey bees.

Studies published between 2015-2018 provide evidence that non-target effects of fipronil on bees exposed to fipronil can include impaired motor function, increased susceptibility to pathogens, morphology and development, and decreased reproductive success. de Morais et al. (2018) found that foraging activity and behavior of a stingless bee species was impaired following 6-24 hours of exposure to sub lethal concentrations of fipronil. Similarly, Bovi et al (2018) determined that fipronil was more toxic to bees when administered by contact than by ingestion, and that both modes of exposure caused bees to take a longer amount of time to navigate through a 50-cm track. In direct contrast, Lunardi et al. (2017) found that Africanized honey bees exposed to fipronil through ingestion experienced impaired motor abilities whereas bees exposed through contact did not exhibit motor changes. These studies do not describe whether or not these concentrations are likely to be encountered by bees in nature.

Kairo et al. (2017a) found that the effect of the fungal parasite *Nosema ceranea* and fipronil exposure alone had negligible effects on bee survival, maturity rates, semen volume, or sperm count , but that when both the fungal parasite and fipronil were added together bee survival dropped from approximately 70% to 40%. Physiological markers in semen were impacted by fipronil alone and in combination with the parasite suggesting that semen quality is affected by the pesticide. In a separate study, Kairo et al (2017b) evaluated the effects of fipronil on honeybee drone fertility and found that drone survival, maturity, and semen volume were not affected by fipronil. However, fipronil did decreased the amount of sperm in semen and increased sperm mortality rate. Collectively these studies suggest that fipronil exposure can influence honeybee reproduction that could potentially contribute to population declines of honeybees.

Munoz-Capponi et al. (2018) evaluated the effects of 6 months of fipronil exposure on honey bee morphology and development. The fipronil treated bees had abnormal development of wings and antennae and smaller body sizes relative to the untreated control group. Zaluski et al. (2017) evaluated the effect of field-relevant doses of fipronil alone and combined with another pesticide, pyraclostrobin, on mandibular and hypopharyngeal glands in nurse honeybees. Bees exposed to fipronil with and without pyraclostrobin had nearly 50% smaller mandibular glands. The pesticides did not influence the number of acini in the hypopharyngeal glands. The amount of brood and worker eggs were also not influenced by the pesticides and no pesticide residues were found in control patties. Though not all parameters measured responded negatively to fipronil exposure, many important physiological, morphological, and developmental traits were impacted. Together these studies provide further evidence that fipronil exposure can impact the health of bees.

Birds

There were two studies published between 2015-2018 that considered the non-target effects of fipronil on bird health. Khalil et al (2017) evaluated the effects of fipronil on Japanese quail reproduction. They tracked quail recovery for 60 days following fipronil exposure. Fipronil reduced food consumption and body weight, and overall had negative effects on reproductive traits in the male quail. However, most of the fipronil impacts on reproductive traits recovered over 20-40 days following exposure. The birds were fed corn oil dosed with 1/5 the lethal dose (LD50) of fipronil. This dose is quite high and not likely encountered. Lopez-Anita et al. (2015) fed fipronil treated and untreated seed to red legged partridges, a farmland associated bird, and compared bird fecundity and offspring quality. Birds did not reject the treated seed, but did reduce the amount consumed. Consumption of fipronil treated seeds resulted in reduced coloration, body condition, and reproductive performance in adult birds and lower quality offspring.

5.3.3 Environmental fate

Between 2015-2018 17-studies were published regarding the persistence and fate of fipronil in the environment. However, all of these studies evaluated intensive use of fipronil in agricultural and residential applications. None of these studies describe the fate of fipronil entering or persisting in the environment through the use of indoor bait traps and are therefore not described here.

5.4 INDOXACARB

Indoxacarb is the active ingredient in Avion gel bait traps used to control cockroaches and ants indoors. It blocks sodium channels and impairs the nervous system and causes paralysis and then death.

5.4.1 Human health risks

No studies were published between 2015 and 2018 that evaluated human health risks associated with indoxacarb exposure.

5.4.2 Ecological Risks

Like fipronil, indoxacarb bait traps are only used by MROSD to control ants or cockroaches inside buildings. The Advion bait traps used by MROSD contain 0.5 g of a formulation that contains 0.6% of the insecticide and they are generally placed strategically >10 feet part, so the potential for exposure of non-target organisms is negligible. No studies published between 2015 and 2018 evaluated the effects of indoxacarb on non-target organisms when indoxacarb is used in bait traps to control indoor pests. Eight studies evaluated lethal and non-lethal effects of indoxacarb on non-target organisms including bees, fish, phytoplankton, arthropods, and snakes (Pozzebon et al. 2015, Neuman-Lee et al. 2016, de Araujo et al. 2017, Fan et al. 2017, Ghelichpour et al. 2017, Li et al. 2018a, Mirghaed et al. 2018, Pashte and Patil 2018). However these studies all evaluate the risks associated with agricultural applications where the pesticide is sprayed onto fields of crop plants to control unwanted pests such as Lepidopteran and Hemipteran herbivores. Because these types of exposure scenarios are irrelevant to MROSD , they are not included in the report, but are described in the annotated bibliography (Appendix 1).

5.5 PHENOTHRIN

No studies were published between 2015 and 2018 that describe human health risks, ecological effects, or the environmental fate of phenothrin.

5.6 PRALLETHRIN

5.6.1 Human health risks

Na et al. 2018 found that prallethrin can cause inflammation in the human respiratory system following inhalation and suggest that adequate ventilation should be required when using indoor insecticides.

5.6.2 Ecological Risks

Two studies were published between 2015-2018 that considered non-target effects of prallethrin on honeybee health. Pokhrel et al. (2018) found that prallethrin applications in a suburban setting had no effect on honeybee mortality, colony health, or production of detoxification enzymes. In a complementary study, Rinkevich et al (2017) also evaluated the effects of prallethrin on honey bee health. Rinkevich et al compared honeybee mortality at six distances up to 91.4 meters from a truck-mounted ultra-low-volume sprayer and found that honeybee mortality was 2% or less with no significant effect of distance from the truck-mounted sprayer.

5.6.3 Environmental fate

In a piolet study, Hung et al. (2018) measured the presence and concentrations of insecticide residues in dust samples collected inside and outside of 56 homes. Prallethrin was found in 38.5 % of the indoor dust samples and in 18% of the outdoor samples. The higher frequency of prallethrin inside suggest that it is or was used inside of homes to control pests.

5.7 S-HYDROPRENE

No studies were published between 2015 and 2018 that describe human health risks, ecological effects, or the environmental fate of S-Hydroprene.

5.8 SODIUM TETRABORATE DECAHYDRATE

No studies were published between 2015 and 2018 that describe human health risks, ecological effects, or the environmental fate of sodium tetraborate decahydrate.

6.0 RODENTICIDES

6.1 CHOLECALCIFEROL

No studies were published between 2015 and 2018 that describe human health risks, ecological effects, or the environmental fate of cholecalciferol.

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Williams, G. M., M. Aardema, J. Acquavella, S. C. Berry, D. Brusick, M. M. Burns, J. L. V. de Camargo, D. Garabrant, H. A. Greim, L. D. Kier, D. J. Kirkland, G. Marsh, K. R. Solomon, T. Sorahan, A. Roberts, and D. L. Weed. 2016. A review of the carcinogenic potential of glyphosate by four independent expert panels and comparison to the IARC assessment. Critical Reviews in Toxicology **46**:3-20.

Yahnke, A. E., C. E. Grue, M. P. Hayes, and S. Pearman-Gillman. 2017. Effects of the herbicide triclopyr on metamorphic northern red-legged frogs. Environmental Toxicology and Chemistry **36**:2316-2326.

APPENDIX A: ANNOTAED BIBLIOGRAPHY

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1.0 HERBICIDES

1.1 GLYPHOSATE

Human Health

Andreotti, G., S. Koutros, J. N. Hofmann, D. P. Sandler, J. H. Lubin, C. F. Lynch, C. C. Lerro, A. J. De Roos, C. G. Parks, M. C. Alavanja, D. T. Silverman, and L. E. B. Freeman. 2018. Glyphosate Use and Cancer Incidence in the Agricultural Health Study. Jnci-Journal of the National Cancer Institute **110**:509-516.

Andreotti et al. (2018) analyzed data from the Agricultural Health Study (AGH). The AGH evaluates health risks associated with agricultural practices, it began in 1993 and includes 54,251 pesticide applicators, 44,932 of which use glyphosate with varying regularity. The initial cancer risk evaluation from this study was conducted in 2005 and at that time 2088 participant had been diagnosed with cancer. In 2005, no significant relationship was found between glyphosate use and cancer, including non-Hodgkin's lymphoma, but there was an increased risk, though not statistically significant, of multiple myeloma in the highest exposure group. Andreotti et al. (2018) is an update to the 2005 evaluation of cancer risk associated with glyphosate, and includes the an additional 7290 cancer cases. This follow-up analysis also includes a questionnaire that participants completed to better describe their glyphosate exposure intensity and frequency and to add additional lifestyle and demographic information. Participants in this study reported the number of years, and an estimated number of days per year that they used glyphosate. This frequency of exposure information was combined with information about how intensive the use was. The intensity of use is related to the amount of chemical the participant can potentially contact. For example, whether the participant mixed or applied pesticides, repaired pesticide-related equipment, used personal protective equipment, and application method used will influence the intensity of the exposure. This information was used to determine glyphosate exposure metrics. Andreotti et al.(2018) concludes that ,no strong evidence was found that glyphosate use increases the risk of cancer, specifically (solid tumors or lymphoid
Malignancies, including Non-Hodgkin's lymphoma. However, they did find some preliminary and not statistically significant evidence of a possible association between myeloid leukemia and glyphosate use for the proportion of participants that had highest intensive of use.

Caballero, M., S. Amiri, J. T. Denney, P. Monsivais, P. Hystad, and O. Amram. 2018. Estimated Residential Exposure to Agricultural Chemicals and Premature Mortality by Parkinson's Disease in Washington State. International Journal of Environmental Research and Public Health **15**.

Parkinson's rates in Washington State are among the highest in the nation, and are 14 percent greater than the national average. Caballero examined the relationship between residential agriculturally-related exposure to glyphosate and Parkinson's-related mortality in Washington State. The study used spatial data to determine the distances between the homes of people who died prematurely from Parkinson's and agricultural land where glyphosate is used. They found that residential exposure to agricultural land use associated with glyphosate had 33% higher odds of premature mortality from Parkinson’s than those who were not exposed.

Davoren, M. J., and R. H. Schiestl. 2018. Glyphosate-based herbicides and cancer risk: a post-IARC decision review of potential mechanisms, policy and avenues of research. Carcinogenesis **39**:1207-1215.

Daveoren and Schiestl (2018) is an excellent non-biased review that desribes the state of the science regarding the health risks of glyphosate debated amoung scientist.

Greim, H., D. Saltmiras, V. Mostert, and C. Strupp. 2015. Evaluation of carcinogenic potential of the herbicide glyphosate, drawing on tumor incidence data from fourteen chronic/carcinogenicity rodent studies. Critical Reviews in Toxicology **45**:185-208.

Greim et al. (2015) obtained and evaluated fourteen proprietary studies conducted on lab rats or mice that investigated the carcinogenicity of glyphosate. These studies were initially performed by Monsanto, the manufacturer of Roundup. and were used by the EPA to inform the decision that glyphosate does not pose significant cancer risk. Upon review Greim et al. (2015) agree with the initial interpretation of the studies and conclude that the studies show no evidence of a carcinogenic effect of glyphosate.

Guyton, K. Z., D. Loomis, Y. Grosse, F. El Ghissassi, L. Benbrahim-Tallaa, N. Guha, C. Scoccianti, H. Mattock, K. Straif, and W. Int Agcy Res Canc Monog. 2015. Carcinogenicity of tetrachlorvinphos, parathion, malathion, diazinon, and glyphosate. Lancet Oncology **16**:490-491.

Guyton et al. (2015) is a summary of the evidence that the International Agency for Research on Cancer (IARC) panel considered when they assessed the carcinogenicity of glyphosate in 2015. Upon review of the following studies the panel classified glyphosate as “probably carcinogenic to humans”. They determined that there was limited evidence in humans for the carcinogenicity of glyphosate. Three case-control studies of occupational exposure of glyphosate reported increased risks for non-Hodgkin lymphoma. The Agricultural Health Study did not show a significantly increased risk of non-Hodgkin lymphoma. In male mice, glyphosate induced a positive trend in the incidence of a rare type of tumor (renal tubule carcinoma). A second study reported a positive trend for sarcoma in male mice. Glyphosate increased pancreatic cell adenoma in male rats in two studies. A glyphosate formulation promoted skin tumors in an initiation-promotion study in mice. Glyphosate has been detected in the blood and urine of agricultural workers, indicating absorption. Blood AMPA (a degradation product of glyphosate) detection after poisonings suggests intestinal microbial metabolism in humans. Glyphosate and glyphosate formulations induced DNA and chromosomal damage in mammals, and in human and animal cells in vitro. One study reported increases in blood markers of chromosomal damage (micronuclei) in residents of several communities after spraying glyphosate formulations. Bacterial mutagenesis tests were negative.

IARC. Some organophosphate insecticides and herbicides. In: IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Lyon: International Agency for Research on Cancer; 2015

This document describes in detail the IARC evaluation process used to determine the carcinogenicity classification of glyphosate, and other herbicides reviewed in 2015.

Williams, G. M., M. Aardema, J. Acquavella, S. C. Berry, D. Brusick, M. M. Burns, J. L. V. de Camargo, D. Garabrant, H. A. Greim, L. D. Kier, D. J. Kirkland, G. Marsh, K. R. Solomon, T. Sorahan, A. Roberts, and D. L. Weed. 2016. A review of the carcinogenic potential of glyphosate by four independent expert panels and comparison to the IARC assessment. Critical Reviews in Toxicology **46**:3-20.

Williams et al. (2016) describes the evaluation process that four independent expert panels used to determine that glyphosate is unlikely to pose a carcinogenic risk to humans. The abstract of the manuscript states: “The International Agency for Research on Cancer (IARC) published a monograph in 2015 concluding that glyphosate is “probably carcinogenic to humans” (Group 2A) based on limited evidence in humans and sufficient evidence in experimental animals. It was also concluded that there was strong evidence of genotoxicity and oxidative stress. Four Expert Panels have been convened for the purpose of conducting a detailed critique of the evidence in light of IARC’s assessment and to review all relevant information pertaining to glyphosate exposure, animal carcinogenicity, genotoxicity, and epidemiologic studies. Two of the Panels (animal bioassay and genetic toxicology) also provided a critique of the IARC position with respect to conclusions made in these areas. The incidences of neoplasms in the animal bioassays were found not to be associated with glyphosate exposure on the basis that they lacked statistical strength, were inconsistent across studies, lacked dose-response relationships, were not associated with preneoplasia, and/or were not plausible from a mechanistic perspective. The overall weight of evidence from the genetic toxicology data supports a conclusion that glyphosate (including GBFs and AMPA) does not pose a genotoxic hazard and therefore, should not be considered support for the classification of glyphosate as a genotoxic carcinogen. The assessment of the epidemiological data found that the data do not support a causal relationship between glyphosate exposure and non-Hodgkin’s lymphoma while the data were judged to be too sparse to assess a potential relationship between glyphosate exposure and multiple myeloma. As a result, following the review of the totality of the evidence, the Panels concluded that the data do not support IARC’s conclusion that glyphosate is a “probable human carcinogen” and, consistent with previous regulatory assessments, further concluded that glyphosate is unlikely to pose a carcinogenic risk to humans.”

Bees

APPENDIX A: ANNOTAED BIBLIOGRAPHY

Abraham, J., G. S. Benhotons, I. Krampah, J. Tagba, C. Amissah, and J. D. Abraham. 2018. Commercially formulated glyphosate can kill non-target pollinator bees under laboratory conditions. Entomologia Experimentalis Et Applicata **166**:695-702.

Abraham et al. (2018) evaluated glyphosate toxicity on honey bees (*Apis melifera*) and a native stingless bee (*Hypotrigona ruspolii*) exposed for 24 hours to either the recommended concentration, twice the recommended rate of Sunphosate, a glyphosate-based herbicide used in Ghana. These bee taxa are considered the most important pollinators in African agriculture. For 24 hours bees were placed in cages with potted plants were sprayed with the herbicide treatments. By contacting the freshly sprayed plants bees were contaminated topically and/or orally with the herbicide. Mortality o both species of bees exposed to the recommended concentration of glyphosate was approximately 20% higher that the mortality of bees in the control group, and exposure to twice the recommended concertation resulted in a 70% increase in mortality. In a second experiment the native bee was exposed to filter paper that had been doused in the herbicide. Once the herbicide treated paper was dry it was placed in a cage with the native bees. The recommended glyphosate concentration dried into filter paper did not affect bee mortality, and twice the recommended dose increased be mortality by 5%.

Balbuena, M. S., L. Tison, M. L. Hahn, U. Greggers, R. Menzel, and W. M. Farina. 2015. Effects of sublethal doses of glyphosate on honeybee navigation. Journal of Experimental Biology **218**:2799-2805.

Sol Balbuena et al. (2015) measured the effects of 2.5,5, and 10 mg/L of sugar solution on bee flight patterns. They measure the proportion of bees that flew directly to the hive compared to the proportion that took indirect flight paths and if there were differences in flight times between treated bees and control bees. They found that bees exposed to 10 mg/L of food had longer flights when taking the direct path, but that the lower doses had no effect on flight time. Not that 10 mg/L is ~3x higher concentration than recommended application.

Berg, C. J., H. P. King, G. Delenstarr, R. Kumar, F. Rubio, and T. Glaze. 2018. Glyphosate residue concentrations in honey attributed through geospatial analysis to proximity of large-scale agriculture and transfer off-site by bees. Plos One **13**.

Berg et al 2018 collected honey samples from hives on Kauai and on Molokai islands. These samples were analysed for glyphosate contamination. The presence and abundance of glyphosate in honey sample was spatially analysed to evaluate if land use could preduct the occurance of contamination. The occurance of glyphosate in honey was predominatly in hives near ro agricultural areas on the western side of Kauai. Only agricultural land use and golf courses were associated with glyphosate contamination in honey. Findings from this study suggest that glyphosate use associtaed with natural areas does not contribute to honey contamination on a large scale.

Cebotari, V., I. Buzu, O. Gliga, O. Postolachi, and N. Granciuc. 2018. Content of pesticide residues in the flowers of the acacia and linden trees from the moldavian codri area. Scientific Papers-Series D-Animal Science **61**:235-242.

This is really poorly written paper, partly because of the authors are not English speakers. The poor writing leaves me skeptical on the rigor/quality of peer review that this manuscript received. None the less, the study is simple and the results are somewhat relevant. Cebotari et al. (2018) measured the concentration of pesticide residues in *Robinia pseudoacacia* and *Tilia platyphillos* flowers from a forest that is presumably near to agricultural land. Average concentrations of fipronil residues in *Robinia f*lowers ranged from 00.003 mg/kg and 0.001 mg/kg in *Tilia* flowers. Glyphosate concentrations were 0.25 mg/kg in *Robina* flowers and 1.25 mg/kg in *Tilia* flowers. The detected concentrations of fipronil residues in the tree flowers was from 1.7 to 5 times lower than the maximum limits allowed according to national and EU standards and glyphosate were 1.6 to 8 times lower the than the maximum limits.

Dai, P. L., Z. X. Yan, S. L. Ma, Y. Yang, Q. Wang, C. S. Hou, Y. Y. Wu, Y. J. Liu, and Q. Y. Diao. 2018. The Herbicide Glyphosate Negatively Affects Midgut Bacterial Communities and Survival of Honey Bee during Larvae Reared in Vitro. Journal of Agricultural and Food Chemistry **66**:7786-7793.

To test the effects of glyphosate on larval mortality, development rate, weight, and gut microbiome Dai et al (2018) fed larvae a sugar solution diet with 0, 0.8, 4 or 20 mg/L of glyphosate. The lowest concentration of glyphosate (0.8mg/L) had no effect on larval mortality, but the two higher concentrations increased mortality. Larval development rate was not affected by glyphosate regardless of the concentration.

Fagundez, G. A., D. C. Blettler, C. G. Krumrick, M. A. Bertos, and C. G. Trujillo. 2016. Do agrochemicals used during soybean flowering affect the visits of Apis mellifera L.? Spanish Journal of Agricultural Research **14**.

Fagundez et al. 2016 evaluated honey bee visitation to soybean flowers after being sprayed with glyphosate to determine if glyphosate contaminated flowers repel bees. Polontor observations began one day after soybean fields were sprayed and were done every 4 days until a second round of glyphosate was applied. Bee visitation to soybean flowers was not influenced by glyphosate applications. Time following herbicide application did not influence bee visitation. These findings suggest that bees do not avoid flowers contaminated with glyphosate. However, this is small study and no unsprayed soybean fields were used as controls.

Faita, M. R., E. d. M. Oliveira, V. V. Alves Junior, A. I. Orth, and R. O. Nodari. 2018. Changes in hypopharyngeal glands of nurse bees (Apis mellifera) induced by pollen-containing sublethal doses of the herbicide Roundup (R). Chemosphere **211**:566-572.

Royal jelly synthesized in the hypopharyngeal glands of bees is an essential protein for feeding the colony, especially the queen. Faita et al. (2018)Bees were fed in an apiary with a sugar and pollen solution with and without Roundup. The morphology and histology the hypopharyngeal glands of nursing worker bees were compared. The authors also measured the amount of royal jelly produced by these nurse bees and the proportion consumed by larvae. They found differences in nurse bee hypopharyngeal gland morphology and histology between bees fed the Roundup treatment and the control. However, these differences were only explained qualitatively and no formal measurements were reported and no statistics were used. They did however measure royal jelly weight and the proportion accepted by bee larvae and did use statistics to evaluate the effects of Roundup, and found no difference in the production (weight) of royal jelly or the accepted by bee larvae.

Liao, L. H., W. Y. Wu, and M. R. Berenbaum. 2017. Behavioral responses of honey bees (Apis mellifera) to natural and synthetic xenobiotics in food. Scientific Reports **7**.

To determine if bees can avoid food sources contaminated with glyphosate Liao et al (2017) conducted a feeding choice experiment and offered bees feeders with glyphosate contaminated sugar water or a sugar water control. Foraging bees preferred sugar water with 10 ppb of glyphosate over the sugar water control, but did not discriminate between sugar water dosed with higher glyphosate concentrations the control. The lack of a dose response makes these results somewhat difficult to interpret. However, the bees clearly did not discriminate against glyphosate treated food when given the choice.

Mengoni Gonalons, C., and W. M. Farina. 2018. Impaired associative learning after chronic exposure to pesticides in young adult honey bees. Journal of Experimental Biology **221**.

With a series of lab experiments Mengoni Goñalons et al. (2018) tested the effects of glyphosate alone and glyphosate combined with a neonicotinoid on honeybee mortality, volume of food (sucrose solution) consumption, ability to detect odor, and ability to detect different concentrations of sucrose. Honey bee mortality was not affected by glyphosate or the glyphosate +neonicotinoid additions. Less food was consumed in the glyphosate treatment in one of the 3 trials. The ability to detect odor was not influenced by glyphosate.

Motta, E. V. S., K. Raymann, and N. A. Moran. 2018. Glyphosate perturbs the gut microbiota of honey bees. Proceedings of the National Academy of Sciences of the United States of America **115**:10305-10310.

Motta et al. (2018) evaluated the effects of glyphosate on the 8 most important bacterial species in the gut of bees. These bacteria have an enzyme that promote weight gain and reduce pathogen susceptibility. Motta et al. (2018) found that glyphosate exposure reduced the abundance of these important gut bacteria and that bees exposed to glyphosate are more susceptible to infection by a particular pathogen, *Serratia marcescens*.

Seide, V. E., R. C. Bernardes, E. J. Guedes Pereira, and M. A. Pereira Lima. 2018. Glyphosate is lethal and Cry toxins alter the development of the stingless bee Melipona quadrifasciata. Environmental Pollution **243**:1854-1860.

Seide et al. 2018 investigated the effects of Roundup on a stingless bee in Brazil that is an important agricultural pollinator. The study actually looks at the effect of bt proteins and glyphosate set out to test the effects of Roundup on bee development and movement, but all the bees fed glyphosate died at the larval stage. The stingless bees fed Roundup contaminated food had higher mortality than the control and died much faster than the control. However, the concentration of Roundup fed to the bees was much higher than what the bees would experience in natural field conditions.

Vazquez, D. E., N. Ilina, E. A. Pagano, J. A. Zavala, and W. M. Farina. 2018. Glyphosate affects the larval development of honey bees depending on the susceptibility of colonies. Plos One **13**.

Vazquez et al. (2018) measured the effects of honey bee larvae fed 0, 1.25, 2.5 or 5 mg of glyphosate/L spiked food (commercial royal jelly) on larval mortality, molting, head diameter, and head weight. They additionally evaluated differences in gene expression of the six different colonies used in the study. They found that effects of glyphosate depended upon the colony. My concern with this study is that the data is presented in the most convoluted way. As far as I can tell there is no linear relationship between glyphosate concentration and mortality, molting head diameter or larval weight. They used six different colonies and all response variables depended upon which colony the bee larvae was from. From this study we can conclude that the effect of glyphosate is driven by colony. We can not conclude the overall impact of glyphosate because the analyses do not use colony as random factor and instead do separate analyses for each colony.

BUTTERFLIES

Saunders, S. P., L. Ries, K. S. Oberhauser, W. E. Thogmartin, and E. F. Zipkin. 2018. Local and cross-seasonal associations of climate and land use with abundance of monarch butterflies Danaus plexippus. Ecography **41**:278-290.

Saunders et al (2018) estimated several factor such as climate, water availability, land cover type and county level glyphosate application from 1994-2012 to examine potential factors contributing to Monarch butterfly decline in the Midwest (USA). At the site level they found a negative relationship between monarch abundance and county level glyphosate use on corn and soybean suggesting that glyphosate and the abundance of land in the Midwest used for growing glyphosate resistant corn and soybean are contributing factors to population declines of Monarch butterflies.

AMPHIBIANS

Bach, N. C., D. J. G. Marino, G. S. Natale, and G. M. Somoza. 2018. Effects of glyphosate and its commercial formulation, Roundup (R) Ultramax, on liver histology of tadpoles of the neotropical frog, Leptodactylus latrans (amphibia: Anura). Chemosphere **202**:289-297.

Bach et al. (2018) compared the effects of Roundup Ultramax and pure glyphosate on the microscopic cell structure (histology) in tadpole livers of the neotropical frog, *Leptodactylus latrans*. Frogs were exposed to 4 different Roundup treatments (0.37, 0.74, 2.22 and 5.25 mg/L) and 4 different glyphosate treatments (3, 15, 75, and 300 mg/L). The number of changes in liver cells of the tadpoles were compared to controls. Changes to liver cell structure increased in abundance with exposure to 0.37 mg/L of Roundup, while the other more concentrated Roundup treatments had no effect on liver cell structure. Only the two highest concentrations of glyphosate (75 and 300 mg/L) altered tadpole liver cell structure. Glyphosate exposure only affected liver cells at lowest glyphosate concentration. The authors suggest that this could be because the frogs may have had a hormone response that triggered a detoxification mechanism that was not triggered at higher concentrations. They also measured 3 modes of hepatic liver damage: lipadosis, congestion, and infiltration lesions. These lesions were only associated with two herbicide treatments, the 2.22 mg/L Roundup treatment and 15 mg/L of Glyphosate treatments. There were no dose dependent effects in the liver cells differences.

Bokony, V., B. Uveges, N. Ujhegyi, V. Verebelyi, E. Nemeshazi, O. Csikvari, and A. Hettyey. 2018. Endocrine disruptors in breeding ponds and reproductive health of toads in agricultural, urban and natural landscapes. Science of the Total Environment **634**:1335-1345.

Bokony et al (2018) collected reproductive common toads (*Bufo bufo*) from urban, natural, and agricultural areas and reared them in a common garden. Toads from all habitats had equally high fecundity, fertilization rates, and offspring viability. The offspring from urban and agricultural areas had reduced development rates and lower body mass. There were 41 contaminants in the 12 ponds were the toads were collected. Glyphosate was found in high concentrations in all habitats. This study is really interesting but is about impacts of land use on toad reproduction rather than glyphosate per se and is not suitable for inclusion in the technical report.

Carvalho, W. F., F. C. Franco, F. R. Godoy, D. Folador, J. B. Avelar, F. Nomura, A. D. da Cru, S. M. Teixeira de Saboia-Morais, R. P. Bastos, and D. d. M. E. Silva. 2018. Evaluation of Genotoxic and Mutagenic Effects of Glyphosate Roundup Original (R) in Dendropsophus minutus Peters, 1872 Tadpoles. South American Journal of Herpetology **13**:220-229.

Carvalho et al (2018) collected *Dendropssophus minutus* frog egg masses in a National Park in Brazil and reared them in a lab and exposed them to 0, 0.28, 1.0, 2.0, or 4.0 mg/L of Roundup Original for 96 hours and then measured DNA alterations and damage. Chemicals that bind to DNA can result in DNA breakage, the loss of genetic material, sister chromatid exchanges, and can trigger carcinogenic processes. Carvalho et al (2018) found that the lowest concentration of Roundup exposer (0.28 mg/L) increased DNA damage and that the higher Roundup exposure treatments had no effect on DNA. Glyphosate had no effect on erythrocytic change in the blood cells. These results are challenging to interpret because of a lack of a dose response effect.

Baier, F., E. Gruber, T. Hein, E. Bondar-Kunze, M. Ivankovic, A. Mentler, C. A. Bruehl, B. Spangl, and J. G. Zaller. 2016. Non-target effects of a glyphosate-based herbicide on Common toad larvae (Bufo bufo, Amphibia) and associated algae are altered by temperature. Peerj **4**.

Baier et al (2016) studied larval development of common toads and algal communities in 3 concentrations of glyphosate (0, 1.5, 3.4 mg/L) under two temperature regimes (15° C and 20° C). The study was conducted in a controlled climate chamber. They measured tadpole mortality, body length to width ratio, developmental stage, tail length, and tail deformations (curvature). Tadpole mortality, development, and body length to width ratio were not different in the glyphosate treatments and the control. However, when the tadpoles were reared at 15° C their tail lengths were slightly shorter when exposed to the Roundup treatments compared to the controls. At 15° C. All Roundup treatments resulted in increased frequency of tail curvature. At 20° C glyphosate had no effect on tail curvature. There is little known about the consequences of shortened tail lengths or tail curvature. It has been suggested that shorter tails may reduce the speed that tadpoles can move to escape predation, further studies are needed to confirm the relationship between tail length and agility. The responses to glyphosate are not dose dependent.

Bokony, V., Z. Miko, A. M. Moricz, D. Kruzselyi, and A. Hettyey. 2017. Chronic exposure to a glyphosate-based herbicide makes toad larvae more toxic. Proceedings of the Royal Society B-Biological Sciences **284**.

Bokony et al. (2017) used two separate experiments to evaluate the relationship between glyphosate and the production of an important defense compound produced by common toad tadpoles. This defense compound is toxic to some tadpole predators, such as fish and newts. In the first study they exposed tadpoles to 2 concentrations (2 or 4 mg/L) of the glyphosate containing herbicide in a lab. The herbicide was applied over a nine day period to toads in five different larval developmental stages or for the entire duration of the study (36-61 days). Only tadpoles that were exposed to herbicide the entire duration of the study produced increased concentrations of the defense chemical. In a complementary study Bokony et al. (2017) applied either 2 or 4 mg/L of the herbicide in a outdoor mesocosm experiment. In this experiment both herbicide treatments resulted in large increases in defense chemical production. The negative effects of the herbicide were dose dependent and robust. The ecological implications of increased defense chemical production is unknown. It could be that increased chemical defense will reduce predation from predators sensitive to the chemical. Alternatively, it could make them more vulnerable to predation by invertebrates if the chemical production reduces development and growth.

Burraco, P., and I. Gomez-Mestre. 2016. Physiological Stress Responses in Amphibian Larvae to Multiple Stressors Reveal Marked Anthropogenic Effects even below Lethal Levels. Physiological and Biochemical Zoology **89**:462-472.

Burraco and Gomez-Mestre (2016) evaluated physiological stress responses of spadefoot toad tadpoles in Spain. They measured corticosterone levels, standard metabolic rate, antioxidant enzyme activity, oxidative cellular damage in lipids, and immunological status of tadpoles exposed for 10 days to 0, 1, or 2 or mg/L of glyphosate. The glyphosate treatments had mixed effects on corticosterone production, at 1 mg/L glyphosate increased production by 91%, whereas 2 mg/L of glyphosate had no significant effect. Both 1 and 2 mg/L glyphosate treatments increased tadpole metabolic rate 2.6 and 2.67-fold. Altered corticosterone levels and increased metabolic rate result in increased energy expenditure which could impact tadpole survival and growth although those links were not evaluated in this study. Glyphosate had no effect on tadpole immune system or antioxidant enzyme activity.

Costa, R. N., and F. Nomura. 2016. Measuring the impacts of Roundup Original((R)) on fluctuating asymmetry and mortality in a Neotropical tadpole. Hydrobiologia **765**:85-96.

Costa and Nomura (2015) determined that the 96-hour LC50 of Roundup original on the tadpoles of the neotropical frog *Physalaemus cuvieri* is 2.3 mg/L of Roundup. They also compared total tadpole length, development, and asymmetry in nostril to snout distance and eye width of tadpole exposed to a single application of 2 mg/L of Roundup and a control. Glyphosate had no effect on either development or size but did increase nose to snout and eye width asymmetry. It has been proposed that trait asymmetry can be an indicator of environmental stress. However further research is needed to understand how trait asymmetries influence tadpole survival and population growth.

Dornelles, M. F., and G. T. Oliveira. 2016. Toxicity of atrazine, glyphosate, and quinclorac in bullfrog tadpoles exposed to concentrations below legal limits. Environmental Science and Pollution Research **23**:1610-1620.

Dornelles and Oliveira (2016) examined the impacts of 0.018 mg/L of glyphosate on several biochemical parameters, and size and mortality of bullfrogs in Brazil. They measured glycogen, triglycerides, total lipids, cholesterol, protein, and lipid peroxidation in the gill, liver and muscle tissues. Glycogen is an immediately available energy source for animals and was found in lower concentrations in the three tissues of bullfrogs exposed to Roundup, suggesting the frogs may have to expend more energy to maintain homeostasis than the control frogs. Total lipids were also found in lower concentrations is the animal tissues with Roundup exposure. This may be because the lipids are being mobilized as a stress response. The biochemical changes due to glyphosate exposure suggest the frogs were experiencing stress. Bullfrog growth (weight and length) and mortality was not affected by Roundup.

Boone, M. D. 2018. An amphibian with a contracting range is not more vulnerable to pesticides in outdoor experimental communities than common species. Environmental Toxicology and Chemistry **37**:2699-2704.

Boone (2018) measured tadpole survival and weight of Blanchard’s cricket frog, gray tree frogs, and green frogs exposed to 1 mg/L of Roundup. Roundup had no effect on tadpole survival or mass for the 3 North American frog species.

Gungordu, A., M. Uckun, and E. Yologlu. 2016. Integrated assessment of biochemical markers in premetamorphic tadpoles of three amphibian species exposed to glyphosate- and methidathion-based pesticides in single and combination forms. Chemosphere **144**:2024-2035.

Gungordu et al. (2016) evaluated the effects of 0.8, 1.6, 3.2, 6.4 and 12.8 mg/L of glyphosate on 3 anuran (aka frog) species. They measured the activity of 6 different biochemical enzymes and combined these data into an “integrated biomarker response”. No dose response effects were found for any of the 7 enzymes measured and the integrated biomarker responses of frogs exposed to glyphosate were not different from the control. The paper does not address how these enzymes contribute to frog survival or fitness.

Krynak, K. L., D. J. Burke, and M. F. Benard. 2017. Rodeo (TM) Herbicide Negatively Affects Blanchard's Cricket Frogs (Acris blanchardi) Survival and Alters the Skin-Associated Bacterial Community. Journal of Herpetology **51**:402-410.

Krynak et al (2017) measured the effects of .75, 1.5, 1.5 and 2.5 mg/L of Rodeo exposure to survival and growth of Blanchard’s cricket frog tadpoles and post metamorphic juvenile frogs. The highest concentration of Rodeo reduced tadpole survival by 37%. They also measured two immune defense traits: the skin associated bacterial community and peptide excretions. The bacterial communities on the frogs treated with the highest concentration of Rodeo were somewhat different from the control. Peptide excretion did not differ from controls. In vitro they also evaluated how the bacterial communities and peptide excretions on the frogs treated with different concentrations of Rodeo inhibited Bd (cytrid fungus) and found no difference across Rodeo treatments and the control. Being exposed to Rodeo as a tadpole did not affect size, mortality, or immune defense traits of post metamorphic juvenile adults.

Levis, N. A., and J. R. Johnson. 2015. Level of UV-B radiation influences the effects of glyphosate-based herbicide on the spotted salamander. Ecotoxicology **24**:1073-1086.

Levis and Johnson (2015) evaluated the interacting effects of low and moderate levels of light availability and a 3 mg/L application of a generic glyphosate herbicide (GLY-4) influence spotted salamander tadpole survival. Tadpole survival was not affected by herbicide in either the low or high light conditions. The proportion of tadpoles that metamorphosed increased with herbicide exposure under both light regimes, suggesting that the herbicide was a stressor to the tadpoles. Cellular immune responses were also measured. At moderate light levels glyphosate decreased the immune response of the tadpole, but at low light glyphosate did not affect the immune response. The findings taken together suggest that glyphosate has a greater impacts on spotted salamander tadpoles in low light environments than out in the open.

Miko, Z., J. Ujszegi, Z. Gal, Z. Imrei, and A. Hettyey. 2015. Choice of experimental venue matters in ecotoxicology studies: Comparison of a laboratory-based and an outdoor mesocosm experiment. Aquatic Toxicology **167**:20-30.

Miko et al. (2015) compared the effects of 0, 2, and 6.5 mg/L of Glyphogan Classic to Agile frog tadpoles in lab and mesocosm study environments. The outcomes of herbicide impact studies done in a lab and mesocosms could be different for a number of reasons including different rates of chemical degradation, indirect effects masked in lab studies, herbicide stratifying in water due to temperature and pH differences between the environments. In this study 6.5 mg/L of the glyphosate herbicide caused 100% mortality of agile frog tadpoles in the lab, but had no effect on mortality in the mesocosm. The 2 mg/l dose of glyphosate also had large impacts on tadpole survival in the lab and not the mesocosm. Similarly, 2 mg/L glyphosate reduced body size in the lab, but had the opposite effect and increased body size in the mesocosm. Glyphosate caused tadpoles to develop faster in the lab and slower in the mesocosm. This study is important because it demonstrates how lab and mesocosm studies can have strikingly different outcomes.

Miko, Z., J. Ujszegi, and A. Hettyey. 2017. Age-dependent changes in sensitivity to a pesticide in tadpoles of the common toad (Bufo bufo). Aquatic Toxicology **187**:48-54.

Miko et al. (2017) exposed the common toad (*Bufo bufo*) to 0, 2, or 4 mg/l of Glyphogan, a glyphosate based herbicide widely used in Europe. The toads were either exposed to the herbicide at the 1st, 2nd, 3rd, 4th, or 5th larval developmental stage and either exposed to the herbicide just once or had sustained exposure. Tadpole survival, time till metamorphose and body mass were measured. At all developmental stages 2 mg/L of the herbicide had no effect on tadpole survival. Tadpoles in the 1st and 2nd development stages or tadpoles that had sustained exposure to 4 mg/L of the herbicide treatment had reduced survival. Tadpole body mass was lower for both the continued exposure herbicide treatments for both the 2 and 4 mg/l concentrations. Body mass was lower in some developmental stages (1st, 4th, and 5th), but not others (2nd, and 3rd) when exposed to the 4 mg/L herbicide. The single application of the low dose (2 mg/L) did not affect tadpole body mass. Time till metamorphosis was 13% and 33% longer for tadpoles exposed to 2 and 4 mg/L for the duration of the study. Only tadpoles in the 1st developmental stage had longer time to metamorphosis with the single application of 2 mg/L of herbicide. Tadpoles at the 1st, 2nd, 3rd, 4th development stage that received 4mg/L of herbicide had longer time till metamorphose. The tadpoles at the 5th development stage developed at the same rate as the control. These results suggest that development of younger frogs may be more sensitive to glyphosate than older frogs.

Moore, H., D. P. Chivers, and M. C. O. Ferrari. 2015. Sub-lethal effects of Roundup (TM) on tadpole anti-predator responses. Ecotoxicology and Environmental Safety **111**:281-285.

Moore et al (2015) measured changes in the amount of movement of wood frog tadpoles when exposed to predator cues and 0.5 mg/L of Roundup Weathermax. Tadpoles reduced activity when they perceive risk of predation. In this study, wood frog tadpoles did not reduce movement when exposed to both chemical predator cues and Roundup. This study used a very realistic concentration of herbicide and provides evidence that real world applications of Roundup can increase tadpole risk of predation.

Soloneski, S., C. R. de Arcaute, and M. L. Larramendy. 2016. Genotoxic effect of a binary mixture of dicamba- and glyphosate-based commercial herbicide formulations on Rhinella arenarum (Hensel, 1867) (Anura, Bufonidae) late-stage larvae. Environmental Science and Pollution Research **23**:17811-17821.

Soloneski et al (2016) evaluated the 96-hour LC50 of Credit, a glyphosate based herbicide used in Argentina that does not contain POEA for tadpoles of a common South American toad, *Rhinella arenarum*. They additionally measured DNA damage accrued in toad cells with 3.91 and 7.82 mg/L Credit exposure. The proportion of cells with damaged DNA increased by 55% with exposure to both concentrations of herbicide. The 96 hour LC50 was 78.18 mg/L.

Relyea, R. A. 2018. The interactive effects of predator stress, predation, and the herbicide Roundup. Ecosphere **9**.

Relyea (2018) investigated how glyphosate toxicity is affected by the presence of predator cues, and lethal predators. In an outdoor artificial pond experiment Relyea (2018) measured the survival of 3 species of amphibian tadpoles (bull frogs, green frogs, gray tree frogs). In each pond 90 tadpoles (30/species) were exposed to 0, 1, 2, or 3 mg/L of Roundup and either live dragonflies (predator presence), live dragonflies in floating cages (predator cues), or empty cages with no predators inside. The predators ate all the frogs in all ponds, suggesting that in the presence of predators the concentration of roundup in the water may be inconsequential to tadpole survival. When caged predators were present increased herbicide concentrations caused little to no reduction in tadpole mortality, depending on the species.

Rissoli, R. Z., F. C. Abdalla, M. J. Costa, F. T. Rantin, D. J. McKenzie, and A. L. Kalinin. 2016. Effects of glyphosate and the glyphosate based herbicides Roundup Original (R) and Roundup Transorb (R) on respiratory morphophysiology of bullfrog tadpoles. Chemosphere **156**:37-44.

Rissoli et al. (2016) compared the effects of pure glyphosate with Roundup Original and Transorb Roundup, on skin morphology and metabolic rates of bullfrog tadpoles. Tadpoles were exposed to 1 mg/L of the herbicides. Skin of many amphibians is an important gas exchange surface. The structure of the skin is very delicate so that there is a short diffusion distance for oxygen to move from water into blood. Skin thickness increased with exposure to glyphosate and Roundup original compared to the control group and Transorb had no effect on skin thickness. Glyphosate reduced metabolic rate during hypoxia, while Transorb increased metabolic rate, and the metabolic rate of the tadpoles exposed to Roundup was not different than the control. Together these results suggest that surfactants and inert chemicals in glyphosate formulations can impact amphibian health.

Ujszegi, J., Z. Gal, Z. Miko, and A. Hettyey. 2015. No observable effect of a glyphosate-based herbicide on two top predators of temporal water bodies. Environmental Toxicology and Chemistry **34**:307-313.

In a 17 day mesocosm experiment (Ujszegi et al. 2015) evaluated survival, behavior, body mass and predatory activity pf 2 aquatic predator species were exposed to 6.5 mg/L of Glyphogan Classic, a glyphosate herbicide that is widely used in Europe. The two species used were the smooth newt (amphibian) and a dragon fly larvae (insect). Survival, behavior, body mass and predatory activity were compared between the animals exposed to the herbicide and a control. No differences in the response factors were detected.

Ujszegi, J., Z. Gal, Z. Miko, and A. Hettyey. 2016. No effect of a glyphosate-based herbicide on larval dragonflies (aeshna cyanea) and adult newts (lissotriton vulgaris) in a laboratory-based experiment. Acta Zoologica Academiae Scientiarum Hungaricae **62**:355-367.

This study was a follow up to Ujszegi et al 2015 (described above) and was very similar. The authors evaluated the effect of Glyphogan Classic, a glyphosate herbicide on dragonfly larvae and smooth newts. Both species are important predators that can exert top-down control of ephemeral lake ecosystems. The differences between this study and the 2015 study is that this study was done in a lab rather than an outdoor mesocosm and the concentration of herbicide was maintained at 6.5 mg/L the entire duration of the study rather than letting it degrade after the initial application. Despite these experimental differences the results were the same. There were no measurable effects of the herbicide on survival, activity, body mass or predatory activity for either predator that was exposed to the herbicide.

Note: The herbicide concentration exceeds what is found in actual water bodies.

Vincent, K., and C. Davidson. 2015. The toxicity of glyphosate alone and glyphosate-surfactant mixtures to western toad (Anaxyrus boreas) tadpoles. Environmental Toxicology and Chemistry **34**:2791-2795.

Vincent and Davidson (2015) determined the 24 and 48 hour LC50 for glyphosate alone (Aquamaster), glyphosate mixed with with Agri-dex or Competitor surfactants for Western toad tadpoles. This study occurred in the San Francisco Bay area. The 24 hour LC50 for glyphosate alone was 8279 mg/L. Glyphosate mixed with Agri-dex had a 24 hour LC50 at 5092 mg/L and glyphosate mixed with the Competitor was 853 mg/L. Glyphosate mixed with Competitor was 6 times more toxic than Glyphosate mixed with Agri-dex and both mixtures were more toxic than glyphosate alone. Glyphosate and glyphosate mixed with Agri-dex have relatively low toxicity. The LC50s in this study are several orders of magnitude above estimated environmental concentrations allowed by the EPA (3.72 mg/L).

Wagner, N., M. Veith, S. Loetters, and B. Viertel. 2017. Population and life-stage-specific effects of two herbicide formulations on the aquatic development of European common frogs (Rana temporaria). Environmental Toxicology and Chemistry **36**:190-200.

Wagner et al. 2017 measured the impacts of Roundup UltraMax on embryo and tadpole survival, growth, and deformity on African clawed frogs and Mediterranean painted frogs. The African clawed frog embryos were exposed to 4.5, 9, 18, 36, and 45 mg/L of herbicide and the tadpoles were exposed to .9, 1.8, 3.6, 4.5, and 9 mg/L. Mortality of African clawed frog embryos increased with 36 and 45 mg/L exposure to Roundup, but not the 4.5, 9, or 18 mg/L treatments. African clawed frog tadpole mortality was not influenced by Roundup, but the tadpoles exposed to 4.5 and 9 mg/L were smaller than the controls. Mediterranean painted frog embryos and tadpoles were exposed to even higher concentrations of Roundup. Embryos were exposed to 45, 90, 135, 180, and 225 mg/L of Roundup and the tadpoles were exposed to 4.5, 9 18 and 36 mg/L. The two largest Roundup doses (180 and 225 mg/L) increased Mediterranean painted frog mortality and only 36 mg/L of Roundup treated tadpoles resulted in higher mortality than the control group.

Wagner, N., H. Mueller, and B. Viertel. 2017a. Effects of a commonly used glyphosate-based herbicide formulation on early developmental stages of two anuran species. Environmental Science and Pollution Research **24**:1495-1508.

Wagner et al. (2017) exposed 3 populations of European common frogs to 2.25, 4.5, 9, 11.25 and 13 mg/L of Roundup UltraMax and compared how the populations varied in survival, growth and deformation to look for a signal of local adaptation to glyphosate. It strikes me as a first pass approach to address this interesting question. The authors only used two populations from forest naïve to glyphosate and 1 population from a pond in an agricultural area. To really determine if local adaptation was occurring they would have needed a few populations for both types of sites (herbicide exposed and herbicide naïve). Tadpole mortality increased in all 3 populations with the 13.5 mg/L Roundup dose and the two forest populations experienced increased mortality at 11.25 mg/L while the population from the agricultural area did not. Tadpole length was shorter for all three populations with the 11.25 and 13 mg/L dose of glyphosate. The 9 mg/L dose of Roundup resulted in shorter tadpoles for the two forest populations, but not the population form the agricultural area. In the agricultural area only the highest concentration resulted in more malformations than the control. The 11.25 mg/L treatment also increased the frequency of malformations in the forest populations and one of the forest populations also had increased malformations with the 9 mg/L treatment. The concentrations used in this study far exceed those found in natural environments.

REPTILES

Burella, P. M., L. M. Odetti, M. F. Simoniello, and G. L. Poletta. 2018. Oxidative damage and antioxidant defense in Caiman latirostris (Broad-snouted caiman) exposed in ovo to pesticide formulations. Ecotoxicology and Environmental Safety **161**:437-443.

Burella et al. (2018) investigated the impacts of embryonic glyphosate exposure on oxidative damage and antioxidant defense in broad-snouted caimen (*Caiman latirostris*).Two formulations of glyphosate-based herbicides were applied to caiman eggshells at a rate of 500, 750, 1000 μg/egg. Oxidative stress caused by glyphosate was not evident at the enzymatic level, but damage to lipids was, this suggests that the antioxidant defense mechanism was impaired. The herbicides did not affect caiman egg viability or hatchling size.

Weir, S. M., S. Y. Yu, A. Knox, L. G. Talent, J. M. Monks, and C. J. Salice. 2016. Acute toxicity and risk to lizards of rodenticides and herbicides commonly used in New Zealand. New Zealand Journal of Ecology **40**:342-350.

Weir et al. (2016) evaluated the toxicity of several herbicides and pesticides at varying concentrations on western fence lizards (*Sceloporus occidentalis*). The chemicals evaluated included 5 rodenticides: brodifacoum, coumatetralyl, pindone, diphacinone and cholecalciferol, and five herbicides: glyphosate, clopyralid, triclopyr, metsulfuron-methyl and haloxyfop-methyl. Here I only describe the results of chemicals included in the MROSD invasive species management plan. Pesticide risk was determined by comparing the 96-hour LD50s to oral exposure models, and when exposure exceeds toxicity, this represents risk. The LD50s was > 1750 μg/g for glyphosate and clopyralid, 550 for triclopyr, and > 1750 for Cholecalciferol. It is important to note that lizard toxicity observed from triclopyr, the toxicity values were still quite high (LD50 = 550 μg g–1) and are probably environmentally unrealistic under normal application scenarios.

Schaumburg, L. G., P. A. Siroski, G. L. Poletta, and M. D. Mudry. 2016. Genotoxicity induced by Roundup (R) (Glyphosate) in tegu lizard (Salvator merianae) embryos. Pesticide Biochemistry and Physiology **130**:71-78.

Schaumburg et al. (2016) evaluated the effects of embryo exposure to Roundup on genotoxicity (DNA damage) of young tegu lizards *(Salvator merianae*). Roundup was applied to lizard eggs at the rate of 50, 100, 200, 400, 800, or 1600 μg/egg. Body weight and snout to vent lengths were measured at birth and again at 6 months and 3 genotoxicity tests (Micronucleus test, Nuclear abnormalities, and a Comet assay) were performed. Glyphosate exposure had no effect on egg viability, birth weight, weigh at six months, or nose to vent length at birth or after 6 months. Of the three test that were performed to evaluate DNA damage, glyphosate exposure increased DNA damage in one test, although this response was not dose dependent. Results from the other two genotoxicity test showed no effect of embryonic glyphosate exposure.

Carpenter, J. K., J. M. Monks, and N. Nelson. 2016. The effect of two glyphosate formulations on a small, diurnal lizard (Oligosoma polychroma). Ecotoxicology **25**:548-554.

Carpenter et al. (2016) evaluated whether dermal exposure to two different commercial glyphosate formulations affected body the body condition of in the New Zealand common skink (*Oligosoma polychroma*). Skinks were sprayed once with Agpro Glyphosate 360, or Yates Roundup Weedkiller, at the recommended rates. Neither glyphosate formulation impacted body weight. Skinks treated with Yates Roundup Weed killer had significantly higher temperatures for 3 weeks following exposure. Carpenter et al. (2016) suggest that increased body temperatures may be a response to increase metabolism and thereby counteract physiological stress.

FISH

Ruiz de Arcaute, C., S. Soloneski, and M. L. Larramendy. 2018. Opposite effects of mixtures of commercial formulations of glyphosate with auxinic herbicides on the ten spotted live-bearer fish Cnesterodon decemmaculatus (Pisces, Poeciliidae). Environmental Pollution **240**:858-866.

Ruiz de Arcaute et al. (2018) determined the acute toxicity of Credit, a glyphosate-based herbicide, on the fish *Cnesterodon decemmaculatus*. The 96 hour LC50 values of for Credit is between 86.80-98.00 mg/L.

Celine Brodeur, J., S. Malpel, A. Belen Anglesio, D. Cristos, M. Florencia D'Andrea, and M. Belen Poliserpi. 2016. Toxicities of glyphosate- and cypermethrin-based pesticides are antagonic in the tenspotted livebearer fish (Cnesterodon decemmaculatus). Chemosphere **155**:429-435.

Brodeur t al. (2016) determined the acute toxicity of Glifoglex, a glyphosate-based herbicide, on the tenspotted livebearer (*Cnesterodon decemmaculatus*) . The 96 hour LC50 value for Glifoglex is 41.4- 53 mg/L. However, toxicity basically reached its maximum
after only 24 hours of exposure.

Goncalves, B. B., N. F. Nascimento, M. P. Santos, R. M. Bertolini, G. S. Yasui, and P. C. Giaquinto. 2018. Low concentrations of glyphosate-based herbicide cause complete loss of sperm motility of yellowtail tetra fish Astyanax lacustris. Journal of Fish Biology **92**:1218-1224.

Goncalves et al. (2018) evaluated the effects of realistic concentrations of glyphosate on sperma motility and viability of yellowtail tetra fish, *Astyanax lacustris* The fish sperm were exposed to 0, 50, 300, and 1800 µg/L of glyphosate. Viability of sperm cells was impaired at 300 µg l−1, and sperm motility was impaired at 50 µg/L, concentrations that are found in waterbodies in the USA.

Jin, J., T. Kurobe, W. F. Ramirez-Duarte, M. B. Bolotaolo, C. H. Lam, P. K. Pandey, T.-C. Hung, M. E. Stillway, L. Zweig, J. Caudill, L. Lin, and S. J. Teh. 2018. Sub-lethal effects of herbicides penoxsulam, imazamox, fluridone and glyphosate on Delta Smelt (Hypomesus transpacificus). Aquatic Toxicology **197**:79-88.

Jin et al (2018) evaluated the effects of glyphosate on the endangered delta smelt (*Hypomesus transpacificus*) in the San Francisco bay. Smelt were exposed to 0.064, 0.64 6.4, 64 and 640 mg/L of glyphosate and E2 and GSH concentrations in the liver and AChE activity in the brain was measured. E2 is a crucial female hormone important to sex maturation. GSH is an indicator of oxidative stress. AchE is an enzyme import to nerve function. Glyphosate exposure increased the E2 hormone in males and had no effect in females. Glyphosate decreased GSH concentrations in males, but had no effect on females. Ache activity in the brain was not effected by glyphosate. Elevated E2 concentrations are problematic, as it can impair the the endocrine system, and reduce reproductive success.

Li, M. H., L. Y. Ruan, J. W. Zhou, Y. H. Fu, L. Jiang, H. Zhao, and J. S. Wang. 2017. Metabolic profiling of goldfish (Carassius auratis) after long-term glyphosate-based herbicide exposure. Aquatic Toxicology **188**:159-169.

The authors exposed goldfish to 34 mg/L of glyphosate for 90 days and found numerous disorders in blood chemistry, and histological evidence of renal damage. Metabolic changes in experimental fish indicated oxidative stress, and several abnormal key metabolic processes. The authors suggest their new approach of integrative metabolomics will be of broad use in understanding the toxic mechanisms of pesticides, even when effects are non-lethal.

Pereira et al. (2018) investigated the neurotoxic effects of Scout, a glyphosate-based herbicide on zebrafish (*Danio rerio*), focusing particularly on changes mitochondria function.

Roy, N. M., B. Carneiro, and J. Ochs. 2016. Glyphosate induces neurotoxicity in zebrafish. Environmental Toxicology and Pharmacology **42**:45-54.

Authors exposed zebrafish to 50 mg/L concentrations of technical grade glyphosate or the Roundup Classic formulation. Both had similar negative effects including morphological anomalies in the head, eyes, and brain. The expression of several genes also decreased in two of three brain regions of exposed fish, as well as gene-receptor changes in the retinal. Authors conclude that both formulations are toxic to affected regions of zebrafish, at least at the one concentration tested.

Zhang, S. H., J. Xu, X. Y. Kuang, S. B. Li, X. Li, D. Y. Chen, X. Zhao, and X. Z. Feng. 2017. Biological impacts of glyphosate on morphology, embryo biomechanics and larval behavior in zebrafish (Dartio rerio). Chemosphere **181**:270-280.

There are a lot of typos and basic spelling and grammatical errors in this paper. How did it get through peer review? Authors document a variety of negative effects of glyphosate exposure at concentrations above 10 mg/L on zebrafish. The described delayed development and increased embryonic death, reuded chorion (outer egg membrane) surface tension, neuronal damage, and increased locomotor activities, among other effects.

MARINE ORGANISMS

Amid, C., M. Olstedt, J. S. Gunnarsson, H. Le Lan, H. Tran Thi Minh, P. J. Van den Brink, M. Hellstrom, and M. Tedengren. 2018. Additive effects of the herbicide glyphosate and elevated temperature on the branched coral *Acropora formosa* in Nha Trang, Vietnam. Environmental Science and Pollution Research **25**:13360-13372.

Amid et al 2018 examined different concentrations of glyphosate on coral bleaching, chlorophyll A content, and the abundance of symbiotic dinoflagellates at current temperatures (28 C) and elevated temperature (31 C). They applied 4 different concentrations of glyphosate (0.12, 1.2, 6.0, and 12. 0 mg/L) to the corals. At 28 C, there was no difference between the glyphosate applications and the control. Regardless of the glyphosate concentration, glyphosate had no effect on bleaching, chlorophyll A, or dinoflagellate abundance at the ambient temperature (28 C). Only corals that received both the highest concentration of glyphosate912 mg/L) and the 3-degree warmer water temperature had increased bleaching, lower chlorophyll A, and fewer dinoflagellates. Note: 12 mg/L of glyphosate exceeds concentrations found in nature.

MACROINVERTEBRATES

Baglan, H., C. R. Lazzari, and F. J. Guerrieri. 2018. Glyphosate impairs learning in Aedes aegypti mosquito larvae a field-realistic doses. Journal of Experimental Biology **221**.

Baglan et al. (2018) tested the impacts of realistic concentrations of glyphosate on the learning ability of mosquito *(Aedes aegypti*) larvae. They exposed larvae to 50, 100, 210 μg/L, and 2 mg/L of glyphosate for the duration of their life. The learning ability of the mosquito larvae was impaired by glyphosate concentrations that could realistically be found in the environment. The higher the glyphosate concentration the larger impact it had on learning. More ecotoxicology studies at these concentrations is needed to really understand the risks of glyphosate-based herbicides to non-target organisms in the environments in which they are applied.

Behrend, J. E., and A. L. Rypstra. 2018. Contact with a glyphosate-based herbicide has long-term effects on the activity and foraging of an agrobiont wolf spider. Chemosphere **194**:714-721.

Behrend and Rypstra (2018) monitored male and egg sac carrying female wolf siders (*Pardosa milvina*) activity and foraging success after 7 hours of exposure to Buccaneer Plus, a glyphosate-based herbicide. The spider offspring had higher levels of activity and greater capture success of prey relative to controls and these effects were larger for males compared to females.

Ferreira-Junior, D. F., R. A. Sarmento, A. d. S. Saraiva, R. R. Pereira, M. C. Picanco, J. L. T. Pestana, and A. M. V. M. Soares. 2017. Low Concentrations of Glyphosate-Based Herbicide Affects the Development of *Chironomus xanthus*. Water Air and Soil Pollution **228**.

Ferreira-Junior et al. (2017) studied the impacts of Roundup original on survival, growth and emergence of *Chironomus xanthas*, a tropical freshwater insect. A 48 LC50 was determined to be 251.5 mg/L of Roundup Original for the larvae. After 10 day of Roundup exposure body length was reduced at the highest exposure level (12.6 mg/L) and all concentrations (0.49, 1.53, 3.69, and 12.06 mg/L) evaluated reduced head size relative to the control. There was no clear dose response effect of the herbicide on emergence.

Hansen, A. A., A. Chatterjee, G. Gramig, and D. A. Prischmann-Voldseth. 2018. Weed and insect management alter soil arthropod densities, soil nutrient availability, plant productivity, and aphid densities in an annual legume cropping system. Applied Soil Ecology **130**:120-133.

More studies like this are needed to understand risks of herbicides relative to other invasive species management methods (e.g. hand pulling, mowing, or not treating, etc.) on non-target organisms. Hansen et al. (2018) compared the effects of Buccaneer Plus, a glyphosate based herbicide, hand-pulling, and leaving the weeds intact on soil properties, growth of target plants, and soil arthropod abundance. Weeding by hand and herbicide had the same impact on soil arthropod abundance. This is an excellent and very nuanced study, but the main take away message is that hand pulling weeds has the same effect on arthropod communities as glyphosate.

Niemeyer, J. C., F. B. de Santo, N. Guerra, A. M. Ricardo Filho, and T. M. Pech. 2018. Do recommended doses of glyphosate-based herbicides affect soil invertebrates? Field and laboratory screening tests to risk assessment. Chemosphere **198**:154-160.

Niemeyer et al. (2018) used both lab and field experiments to test the impacts of four different glyphosate-based herbicides (Roundup Original, Trop, Zapp, and Crucial) on soil macroinvertebrates (earthworms, isopods, collembolans, and bait lamina) avoidance behavior and reproduction. One application of the recommended doses of herbicides did not cause the earthworms, isopods, or collembollas to avoid the sprayed oats. An important strength of this experiment is that the researchers sprayed actual fields of weeds and then observed the macroinvertebrates response rather than only applying high doses in a lab environment that organisms are unlikely to encounter in nature.

Pereira, J. L., T. V. S. Galdino, G. A. R. Silva, M. C. Picanco, A. A. Silva, A. S. Correa, and J. C. Martins. 2018. Effects of glyphosate on the non-target leaf beetle Cerotoma arcuata (Coleoptera: Chrysornelidae) in field and laboratory conditions. Journal of Environmental Science and Health Part B-Pesticides Food Contaminants and Agricultural Wastes **53**:447-453.

Pereira et al. (2018) studied the effect of glyphosate on *Cerotoma arcuta*, a leaf beetle and a crop pest. Soybean fields were sprayed with the recommended dose of Roundup Original. Densities of the beetles and their predators were compared in sprayed fields and control fields where weeds were hand pulled. One application of glyphosate had no effect on beetle densities. Three applications reduced beetle densities. Beetle predators decreased with one application of Roundup. These results suggest that glyphosate may have released the beetle from predation. In a separate lab experiment the beetles were exposed to a Roundup treated and an untreated plant substrate and beetles spent less time on the Roundup treated plants.

Stellin, F., F. Gavinelli, P. Stevanato, G. Concheri, A. Squartini, and M. G. Paoletti. 2018. Effects of different concentrations of glyphosate (Roundup 360 (R)) on earthworms (*Octodrilus complanatus*, *Lumbricus terrestris* and *Aporrectodea caliginosa*) in vineyards in the North-East of Italy. Applied Soil Ecology **123**:802-808.

Stellin et al. (2018) collected two species of earth worms in grasslands not treated with glyphosate and from vineyards that were routinely treated with glyphosate. The worms were reared in terraria and were exposed to 0, 0.59, 2.9, 5.79, or 11.59 g/m^2 of Roundup. In this region in Italy, where this study occurred, the typical Roundup application rates are 0.72 g/m^2 to 4.32 g/m^2. All doses of Roundup increased worm mortality and cocoon production relative to the control and the larger the dose the larger the effects.

von Merey, G., P. S. Manson, A. Mehrsheikh, P. Sutton, and S. L. Levine. 2016. Glyphosate and aminomethylphosphonic acid chronic risk assessment for soil biota. Environmental Toxicology and Chemistry **35**:2742-2752.

Von Merey et al. (2016) evaluated the potential effects of glyphosate and AMPA (a biproduct of glyphosate) on survival and reproduction of detritivores (e.g., earthworms and springtails) and predatory mites. At soil concentrations relevant to recommended glyphosate field application rates, no significant adverse effects were observed in any of the test species exposed to glyphosate or AMPA.

NON-TARGET PLANTS

Belz, R. G., M. Patama, and A. Sinkkonen. 2018. Low doses of six toxicants change plant size distribution in dense populations of *Lactuca sativa*. Science of the Total Environment **631-632**:510-523.

Belz et al. (2018) used a dose response experiment to evaluate sublethal effects of glyphosate (and other pesticides) on lettuce (*Lactuca sativa*) to determine if fast and slow growing individuals within a population are differentially influenced. A possible consequence of differentially effecting fast and slow growing individuals is a change in the size distribution of a population. The authors suggest that during drought events plants with rapid growth will be at an advantage and slower growing plants with short roots could be eliminated due to poor water uptake. The authors further suggest that differential response to glyphosate could lead to enhanced drought resistance. In general, they found that larger plants were more susceptible to non-lethal effects of low dose applications of glyphosate.

Debski, H., M. Szwed, D. Koczkodaj, J. Klocek, and M. Horbowicz. 2018. Comparison of the response of seedlings of common buckwheat (Fagopyrum esculentum Moench) to glyphosate applied to the shoot or to the root zone. Acta Agrobotanica **71**:1730-Article No.: 1730.

In a controlled growth chamber experiment Dębski et al (2018) examined the response of common buckwheat (*Fagopyrum esculentum)* seedlings to 3 doses of a glyphosate based herbicide. Glyphosate is applied to the leaves of target plants. Rain can rinse the glyphosate off the target plants and deposit it onto the soil. Glyphosate applied to the root zone had a weak impact on buckwheat shoot length. Whereas glyphosate applied to the shoots had a large negative effect on buckwheat seedling growth at the highest concentration (169 mg/L.

Glyphosate had idiosyncratic effects on the amount anthocyanins in buckwheat, that is, some doses in some substrates increase anthocyanin production and some concentration decrease production in a non-dose response fashion.

This study is extremely unrealistic. The concentrations of glyphosate used are excessively high and would not be encountered in actual field conditions. The researchers dipped the plant roots into the herbicide, this is also an unrealistic delivery method. In the field herbicide would pass through soil and the soil microbial community which would likely have a diluting effect.

Gomes, M. P., S. G. Le Manac'h, S. Maccario, M. Labrecque, M. Lucotte, and P. Juneau. 2016. Differential effects of glyphosate and aminomethylphosphonic acid (AMPA) on photosynthesis and chlorophyll metabolism in willow plants. Pesticide Biochemistry and Physiology **130**:65-70.

The authors find that both glyphosate and AMPA, the principal by-product of glyphosate decomposition, negatively affected chlorophyll in willows. Interestingly, while both chemicals ultimately negatively affected photosynthesis, the details of how they interfered with chlorophyll concentration are distinct: Glyphosate increased the degradation of chlorophyll, whereas AMPA interfered with chlorophyll biosynthesis. Both chemicals caused the accumulation of reactive oxygen species in willows.

Turedi, M., D. Esen, and B. Cetin. 2018. Seed screening of three pine species for glyphosate sensitivity for forest restoration. Plant Biosystems **152**:502-507.

Turedi et al. (2018) evaluated the toxicity of glyphosate to seeds of three non-target pine species: *Pinus nigra*, *Pinus sylvesteris*, and *Pinus pinaster*. These pines are used in forestry around the world. Although none of the three pines examined in this study are native to California the study is somewhat relevant because the authors demonstrated that seeds of different Pine species can vary in their sensitivity to glyphosate. It may be that seeds of California pines are or are not sensitive to glyphosate and this should be considered if spraying sites where pine regeneration from seed is desired. The seeds in this experiment were soaked in 100 mL Roundup solution which far exceeds the concentration of Roundup seeds on the surface or buried in soil would encounter in actual field applications of herbicide. None the less, this research suggest seeds of different species in a soil seed bank may be differentially influenced by glyphosate. More research is needed to determine if realistic exposure to glyphosate impacts seed germination.

Stark, P. B., D. Miller, T. J. Carlson, and K. R. de Vasquez. 2019. Open-source food: Nutrition, toxicology, and availability of wild edible greens in the East Bay. Plos One **14**.

Stark et al. (2019) considered the risk of glyphosate on toxicity and nutrition of edible naturalized leafy greens that are foraged by the local community members. Leafy greens were collected from 3 separate urban sites in economically disadvantaged neighborhoods in the East San Francisco Bay. In these neighborhoods people forage for wild edibles. Glyphosate was below the detection limits in the plant tissue and the nutritional content was equivalent to the most nutritious commercially available leafy greens.

Reeg, J., S. Heine, C. Mihan, S. McGee, T. G. Preuss, and F. Jeltsch. 2018. Simulation of herbicide impacts on a plant community: comparing model predictions of the plant community model IBC-grass to empirical data. Environmental Sciences Europe **30**.

Reeg et al. (2018) evaluated the value of landscape modeling to predict non-target impacts of glyphosate herbicide on the structure of grassland plant communities that occur near to agricultural fields where glyphosate is routinely used. They compared the model predictions with experimentally measured effects. For the experiment the applied 3, 5, 9, 15, and 25% of the maximum application rate of 3L/ha of Roundup to 6 different grassland plants growing alone or with the other 5 species. The modelling approach used a plant functional type approach and predicts community structure with different disturbance regimes (e.g grazing, herbicide, etc.). The model was calibrated with data from the monocultures in the experiment. They determine that the model effectively predicted the impacts of glyphosate drift on plant community structure.

MYCORRHIZAL FUNGI

Druille, M., M. N. Cabello, P. A. G. Parisi, R. A. Golluscio, and M. Omacini. 2015. Glyphosate vulnerability explains changes in root-symbionts propagules viability in pampean grasslands. Agriculture Ecosystems & Environment **202**:48-55.

Druille et al. 2015 evaluated the effect of glyphosate on the viability of arbuscular mycorrhizal fungal spores and rhizobium propagules in a native grasslands in Argentina. The number of viable arbuscular mycorrhizal spores was reduced with a single application of the recommended rate of Glacoxan, a glyphosate-base herbicide. Viability of the four dominant fungal species was variable following herbicide application, viability declined for two of the AMF species. The community composition of mycorrhizal fungi was also influenced by the herbicide. Glyphosate also reduced the abundance of Rhizobia that associate with legume plants.

Druille, M., M. N. Cabello, P. A. G. Parisi, R. A. Golluscio, and M. Omacini. 2015. Glyphosate vulnerability explains changes in root-symbionts propagules viability in pampean grasslands. Agriculture Ecosystems & Environment **202**:48-55.

Druille et al. (2016) evaluated the impact of four annual glyphosate applications on arbuscular mycorrhizal fungi (AMF), dark septate endophytes (DSE) and free-living diazotrophs in a temperate grassland. AMF viable spores and free-living diazotrophs densities were reduced by 56% and 82% respectively, after the fourth glyphosate application. However, despite less AMF propagule abundance, mycorrhizal root colonization on *Lolium arundinaceum* was not affected by the glyphosate appliactions. Glyphosate also reduced the proportion *Lolium arundinaceum* roots colonized by dark septate endophytes.

Nivelle, E., J. Verzeaux, A. Chabot, D. Roger, Q. Chesnais, A. Ameline, J. Lacoux, J.-E. Nava-Saucedo, T. Tetu, and M. Catterou. 2018. Effects of glyphosate application and nitrogen fertilization on the soil and the consequences on aboveground and belowground interactions. Geoderma **311**:45-57.

This is a multifaceted study that mostly pertains to the interactive effects of nitrogen fertilizer and glyphosate on abiotic and biotic soil properties and crop plant performance. Some of the information however can inform non-agricultural impacts of glyphosate on mycorrhizal abundance and function and soil fertility. Nivelle et al (2018) found that .96 mg/kg of glyphosate had no effect on the abundance of arbuscular mycorrhizal fungi that associate with the non-target crop plant. The herbicide also had no effect on dehydrogenase or alkaline phosphatase activity which are enzymes produced by microbes such as mycorrhizal fungi to release minerals from unusable forms into usable forms. Glyphosate total nitrogen and the carbon to nitrogen ratio. The effect on total nitrogen did not translate to changes in plant nitrogen or the carbon to nitrogen ratio in plant leaf tissue. Glyphosate also did not influence aphid survival.

Helander, M., I. Saloniemi, M. Omacini, M. Druille, J.-P. Salminen, and K. Saikkonen. 2018. Glyphosate decreases mycorrhizal colonization and affects plant-soil feedback. Science of the Total Environment **642**:285-291.

This is a multifaceted study that considers the interactions of tilling, endophyte dependence and glyphosate on a non-target forage grass species. Helander et al. (2018) conducted a pot experiment and grew a Festuca pratensis, a non-target forage grass in soil where a weedy grass was sprayed with 2x the normal dose of Roundup. For the purpose of this review I will only summarize the effects of glyphosate in the absence of tilling. Non-target plant establishment or growth was not influenced by Roundup. There was also no effect of Roundup on soil chemistry (i.e. soil pH, calcium, magnesium, potassium and phosphorus. However, the proportion of roots colonized by arbuscular mycorrhizal fungi decreased on the non-target forage grass grown in the Roundup treated soil.

Jusaitis, M. 2018. Herbicidal control of bridal creeper (Asparagus asparagoides) in an ecologically sensitive environment. Pacific Conservation Biology **24**:3- 11.

Jusaitis (2018) compared the efficacy of spray and wipe applications of glyphosate and metsulfuron methyl, applied separately or together, to control *Asparagus asparagoides* and evaluated the effect of glyphosate on mycorrhizal fungi associated with an endangered orchid (*Pterostylis arenicola*) that co-occurs with the invader. Mycorrhizal fungi were cultured from orchid roots and grown in agar spiked with 0, 0.5, 1.0, 1.5 or 3.0 kg /ha of Roundup. Fungal survival was not influenced by glyphosate. The growth of mycorrhizal fungi decreased with increasing concentrations of herbicide.

Maltz, M. R., C. E. Bell, M. J. Mitrovich, A. R. Iyer, and K. K. Treseder. 2016. Invasive Plant Management Techniques Alter Arbuscular Mycorrhizal Fungi. Ecological Restoration **34**:209-215.

Maltz et al. (2016) compared the effects of glyphosate and mowing an invsive non-mycorhizal plant, Brassica nigra, on hyphal abundance of mycorhizal fungi. Glyphosate increased the abundance of mycorrhizal fungi

Dupont, Y. L., B. Strandberg, and C. Damgaard. 2018. Effects of herbicide and nitrogen fertilizer on non-target plant reproduction and indirect effects on pollination in Tanacetum vulgare (Asteraceae). Agriculture Ecosystems & Environment **262**:76-82.

This implications from this study are difficult to evaluate because of two important issues with the experimental design and data analysis. Dupont et al (2018) conducted a randomized block design experiment and sprayed different concentrations of Roundup to field plots that contained the non-target plant, *Tanacetum vulgare*. The concentrations of Roundup used were selected to mimic the amount of herbicide drift plants in close proximity to agriculture fields might experience. The concentrations used were 0, 1, 5 and 25 % of the label rate. They measured floral density, flowering phenology, capitula diameter, plant height, visitation rates of pollinators, diversity of flower-visitors, and per seed weight as well as total seed weight. There are a few important problems with this study. First, the authors did not describe how the abundance (e.g. percent cover or density) of *Tanacetum* varied across plots to begin with and it could be that the treatment effects are really just due to underlying differences in starting abundance of *Tanacetum*. Pre treatment density is important to know since the authors did not control for abundance by planting *Tanacetum* at the same rate across treatments (e.g. seeds/plot). Another factor contributing to my inability to evaluate the implications is that the authors only report statistics for glyphosate compared to a control and do not address how the 4 different glyphosate treatments varied.

SOIL MICROBIAL COMMUNITIES

Dennis, P. G., T. Kukulies, C. Forstner, T. G. Orton, and A. B. Pattison. 2018. The effects of glyphosate, glufosinate, paraquat and paraquat-diquat on soil microbial activity and bacterial, archaeal and nematode diversity. Scientific Reports **8**.

A single application of the recommended field rate of glyphosate was applied to soil at the recommended doses and the soils were incubated in a lab for 60 days. The abundance, richness, evenness and community composition of nematodes, and bacterial and archaeal soil microbes were measured repeatedly over the 60-day study. The authors also measured fluorescein diacetate hydrolysis (FDA) and beta glucosidase. FDA is an integrated measure of total microbial enzyme activity and beta glucosidase is a rate limiting step in the degradation of cellulose. The recommended application rate of glyphosate had no significant effects on the abundance, richness, evenness, or community composition of nematodes, soil bacteria or archaeal communities, or the enzymatic activity of the soil microbes. These findings suggest that a single application of glyphosate at the recommended dose poses little threat to soil biodiversity and function. One short coming of this study is that the soils were collected from an agricultural area, where glyphosate is routinely used. It is possible that herbicide-intolerant organisms were already depleted.

Hernandez Guijarro, K., V. Aparicio, E. De Geronimo, M. Castellote, E. L. Figuerola, J. Luis Costa, and L. Erijman. 2018. Soil microbial communities and glyphosate decay in soils with different herbicide application history. Science of the Total Environment **634**:974-982.

Hernandez et al. (2018) evaluated the relative importance of soil microorganisms and abiotic soil characteristics of glyphosate degradation in soils that had not previously been exposed to glyphosate, had been exposed repeatedly over 5 years or had been exposed repeatedly over 10 years. They evaluated the degradation rate of glyphosate on these three types of soils. In field soils glyphosate degraded regardless of previous exposure. The degradation of glyphosate did not result in changes in microbial communities, likely because many microbes can use glyphosate as a source of nitrogen, phosphorus, and carbon.

Liu, Y., Y. Lil, X. Hua, K. Muller, H. Wang, T. Yang, Q. Wang, X. Peng, M. Wang, Y. Pang, J. Qi, and Y. Yang. 2018. Glyphosate application increased catabolic activity of gram-negative bacteria but impaired soil fungal community. Environmental Science and Pollution Research **25**:14762-14772.

Liu et al. (2018) sprayed soil one time with the recommended glyphosate application rate or a 10-fold higher rate and evaluated how communities of soil bacteria and soil fungi respond to glyphosate. They also evaluated if changes in bacterial communities resulted in increased catabolic activity of Gramnegative bacteria. Lastly, they evaluated if the EPSPS gene that confers tolerance to glyphosate increases in abundance with glyphosate exposure. Six months after the glyphosate treatments were applied soils were collected and analyzed. Neither the recommended dose or the 10 -fold higher does affected the bacterial communities. The recommended dose of glyphosate did not increase the abundance of the gene that confers glyphosate tolerance while the 10-fold higher dose resulted in higher abundance of the glyphosate tolerance gene. Fungal abundance declined in the soils exposed to both the recommended and 10-fold higher doses of glyphosate.

Wech, J., A. Suren, M. Brady, and C. Kilroy. 2018. The effect of willow control using a glyphosate formulation on aquatic invertebrates within a New Zealand wetland. New Zealand Journal of Marine and Freshwater Research **52**:16-41.

Wech et al. (2018) compared invertebrate assemblages before and after glyphosate was used to treat invasive willows in New Zealand wetlands. Glyphosate reduced willow cover to less than 20%. Invertebrate communities were sampled for 3.5 years following the glyphosate treatment. Invertebrate richness or community structure was not significantly impacted by glyphosate.

Reno, U., S. R. Doyle, F. R. Momo, L. Regaldo, and A. M. Gagneten. 2018. Effects of glyphosate formulations on the population dynamics of two freshwater cladoceran species. Ecotoxicology **27**:784-793.

Reno et al. (2018) experimentally assessed the effects of glyphosate on two non-target freshwater cladoceran species and used this information to predict the population dynamics of these microcrustaceans and the potential for recovery following glyphosate exposure. They determined the 48-hour LC50 for these microcrustaceans for 4 different glyphosate-based herbicides. The 48 hour LC50 for *Daphnia magna* with Eskoba was between 27.464–31.415 mg/L., between 8.93–15.43 for Roundup Ultramax, between 1.68–2.67 with Panzer Gold, and between 1.24–2.09 with Sulfosata Touchdown. *Ceriodaphnia dubia* was more sensitive to all the herbicides demonstrated by lower LC50 values for each herbicide. To determine how these organisms, recover following herbicide exposure the surviving individuals in the LC50 tests were put in glyphosate free containers and survival, number of molts, and fecundity were measured. This demographic data was then included in a population model to evaluate if populations exposed to these concentrations of glyphosate-based herbicides will persist or perish. The model determined that populations of *Daphnia magna exposed to the concentrations used in the LC50 study (described above) would not persist.* Likewise, the concentrations used in the LC50 study for *Ceriodaphnia dubia* will not persist with the exception of Roundup Ultramax. This study approach is very valuable for assessing risks of pesticides. However, one downfall of this study is that the concentrations used far exceed concentrations likely encountered in natural or agricultural areas.

EFFICACY

Caplan, J. S., R. D. Whitehead, A. E. Gover, and J. C. Grabosky. 2018. Extended leaf phenology presents an opportunity for herbicidal control of invasive forest shrubs. Weed Research **58**:244-249.

Caplan et al. (2018) conducted a small proof of concept study to evaluate if extend leaf phenology of *Lonicera maackii* associated with climate change can provide a larger window for effective control with foliar applications of glyphosate. Photosynthesis was measured on green leaves in the early November and compared to published rates for summer. Plants were less photsynthetic than during the standard growing season. Glyphosate was applied in November and treatment effectiveness was determined the following spring by rating cambial mortality. The authors conclude that glyphosate applied in November was highly effective and suggest that other invasive species that maintain green leaves into the fall may be effectively treated outside the typical herbicide application period.

DeGreeff, R. D., A. V. Varanasi, J. A. Dille, D. E. Peterson, and M. Jugulam. 2018. Influence of Plant Growth Stage and Temperature on Glyphosate Efficacy in Common Lambsquarters (Chenopodium album). Weed Technology **32**:448-453.

DeGreeff et al. (2018) evaluated the efficacy of glyphosate to control lambs quarters (*Chenopodium album*) applied to plants at 4 different life stages (i.e. plant heights) and at different temperatures. They determined that control is most effective when applied to the smallest plant (5-7 cm tall) and in the coolest temperatures (25° C day and 15° C at night). They recommend treating lambs quarters with glyphosate early in the growing season, when temperatures are low and plants are still small.

El-Tokhy, A. I. 2018. Efficacy of glyphosate and fluazifop-P-butyl herbicides with adjuvants at different levels of cutting for the common reed (Phragmites australis). Journal of Plant Protection Research **58**:282-288.

El-Tokhy (2018) evaluated the efficacy of glyphosate to control common reed grass (*Phragmites australis*) following cutting to various heights. Twelve months following treatment, glyphosate alone reduced *Phragmites* cover by a little over 80%, while glyphosate with adjuvants decreased *Phragmites* by an additional 10% (i.e. by 90%), and glyphosate applied at half the recommended rate but with adjuvants added was slightly more effective than the full dose applied without adjuvants.

Enloe, S. F., R. D. Lucardi, N. J. Loewenstein, and D. K. Lauer. 2018. Response of twelve Florida cogongrass (Imperato cylindrica) populations to herbicide treatment. Invasive Plant Science and Management **11**:82-88.

Enloe et al. (2018) evaluated the efficacy of controlling 12 independent populations of cogongrass (*Imperata cylindrica*) in Florida with glyphosate (0.28 kg/ha of Rodeo). The effectiveness of glyphosate was similar across all populations and glyphosate alone reduced cogongrass biomass by 78%.

Enloe, S. F., S. E. O'Sullivan, N. J. Loewenstein, E. Brantley, and D. K. Lauer. 2018b. The Influence of Treatment Timing and Shrub Size on Chinese Privet (Ligustrum sinense) Control with Cut Stump Herbicide Treatments in the Southeastern United States. Invasive Plant Science and Management **11**:49-55.

Enloe et al. (2018) evaluated the effectiveness of glyphosate and triclopyr applied to cut stump of Chinese privet (*Ligustrum sinense*) compared with cutting alone at spring and fall timings across a range of size classes. Both glyphosate and triclopyr were very effective in controlling privet at both spring and fall applications to cut stumps. Glyphosate was slightly more effective than triclopyr. Less sprouting occurred following November treatments relative to April treatments. Larger diameter stumps tended to sprout more than smaller stumps. These findings suggest privet can be effectively controlled by applying glyphosate or triclopyr to cut stumps in the spring or fall.

Farthing, T. S., J. P. Muir, A. D. Falk, and D. Murray. 2018. Efficacy of Seven Invasive-Bermudagrass Removal Strategies in Three Texas Ecoregions. Ecological Restoration **36**:306-314.

Farthing et al (2018) evaluated the efficacy of glyphosate and imazapyr at removing Bermudagrass (*Cynodon dactylon*) from grasslands in Texas. Glyphosate applied once reduced Bermudagrass cover by 70%. Two applications of glyphosate reduced bermudagrass cover by >95%. Glyphosate alone was as effective as mowing prior to glyphosate application. Imazapyr applied only once was as effective at controlling Bermudagrass as multiple applications of glyphosate. However, imazapyr's half-life in soil ranges from one to five months and non-target species may be adversely affected.

Griffiths, J., H. Armstrong, R. Innes, and J. Terry. 2018. Can aerial herbicide application control Grey Willow (Salix cinerea L.) and stimulate native plant recovery in New Zealand wetlands? Ecological Management & Restoration **19**:49-57.

Griffiths et al. (2018) compared the efficacy of controlling grey willows in New Zealand wetlands with glyphosate and triclopyr. Glyphosate reduced Grey willow cover which resulted in increased cover of native wetland plants. Triclopyr was much less effective at controlling grey willow than glyphosate and did not result in increased cover of natives.

Guerra-Garcia, A., D. Barrales-Alcala, M. Argueta-Guzman, A. Cruz, M. C. Mandujano, J. A. Arevalo-Ramirez, B. G. Milligan, and J. Golubov. 2018. Biomass Allocation, Plantlet Survival, and Chemical Control of the Invasive Chandelier Plant (Kalanchoe delagoensis) (Crassulaceae). Invasive Plant Science and Management **11**:33-39.

Guerra-Garcia et al. (2018) compared the use of several herbicides alone and in combination to control chandelier plant (*Kalanchoe delagoensis*). Glyphosate applied alone resulted in greater than 85% plant mortality and glyphosate applied in combination with 24-D resulted in 100% mortality.

Jones, D., G. Bruce, M. S. Fowler, R. Law-Cooper, I. Graham, A. Abel, F. A. Street-Perrott, and D. Eastwood. 2018. Optimising physiochemical control of invasive Japanese knotweed. Biological Invasions **20**:2091-2105.

Jones et al. (2018) conducted a large field experiment to evaluate numerous approaches to control Japanese knotweed (*Fallopia japo*nica). No treatment completely eradicated F. japonica, a multiple-stage glyphosate-based treatment approach provided greatest control. The most effective approaches were spraying 2.16 kg/ha in the summer and again the following fall or by spraying only in the fall at a rate of 3.6 kg/ha.

Jusaitis, M. 2018. Herbicidal control of bridal creeper (Asparagus asparagoides) in an ecologically sensitive environment. Pacific Conservation Biology **24**:3- 11.

Jusaitis (2018) compared the efficacy of spray and wipe applications of glyphosate and metsulfuron methyl, applied separately or together, to control *Asparagus asparagoides* and evaluated the effect of glyphosate on mycorrhizal fungi associated with an endangered orchid

(*Pterostylis arenicola*) that co-occurs with the invader. Mycorrhzal fungi were cultured from orchid roots and grown in agar spiked with 0, 0.5, 1.0, 1.5 or 3.0 kg /ha of Roundup. Fungal survival was not influenced by glyphosate. The growth of mycorrhizal fungi decreased with increasing concentrations of herbicide. Six years following the treatments glyphosate + metsulfuron methyl spray and metsulfuron methyl sprayed alone had 29% and 24% cover of the invader. Glyphosate wipe and spray treatments were below 15%. The most lasting control of *A. asparagoides* was the metsulfuron methyl wipe (6%) and the glyphosate + metsulfuron wipe (3%) each of which received only the single initial application of herbicide.

Knezevic, S. Z., O. A. Osipitan, M. C. Oliveira, and J. E. Scott. 2018. Lythrum salicaria (Purple Loosestrife) Control with Herbicides: Multiyear Applications. Invasive Plant Science and Management **11**:143-154.

Knezevic et al. (2018) evaluated the effectiveness of 14 herbicide treatments for purple loosestrife (*Lythrum salicaria*) control over a 10-year period. Here I will describe only the treatments relevant to MROSD. Glyphosate was applied at 2.2 and 3.4 kg/ha, triclopyr at 1.3 and 2.1 kg/ha, and imazapyr at 1.1 and 1.7 kg/ha. The herbicides were applied every year until 100% control was achieved. The high application rates of glyphosate and imazapyr provided excellent purple loosestrife control (≥90%) that lasted at least a year following application, and by year 3 both herbicides had eradicated the invader. It took 9 years to eradicate purple loosestrife with triclopyr. The older the purple loosestrife stand, the more years of follow-up application were needed. Generally, there were higher percentages of grasses following triclopyr applications and higher cover of broadleaf species following glyphosate or imazapyr treatments.

Leahy, M. J., I. W. Vining, J. L. Villwock, R. O. Wesselschmidt, III, A. N. Schuhmann, J. A. Vogel, D. Y. Shieh, and C. J. Maginel, III. 2018. Short-term efficacy and nontarget effects of aerial glyphosate applications for controlling Lonicera maackii (Amur honeysuckle) in oak-hickory forests of Eastern Missouri, USA. Restoration Ecology **26**:686-693.

Leahy et al. (2018) evaluated the short term efficacy of an aerial application of glyphosate in reducing foliar cover and stem density of *Lonicera maackii* in the understory of forest stands and to determine the short-term impacts from this treatment to native overstory and understory tree and shrub stem density and herbaceous and woody ground-layer species cover.The stem density of non-target overstory trees were not influenced by the glyphosate application. One year following the herbicide application Lonicera stem density only declined 42% and cover declined by 78%. Following the herbicide application there was no difference in cover of native understory species, but the abundance of individual species did increase or decrease in response to the herbicide.

Mahmood, A. H., S. Florentine, F. P. Graz, C. Turville, G. Palmer, J. Sillitoe, and D. McLaren. 2018. Comparison of techniques to control the aggressive environmental invasive species Galenia pubescens in a degraded grassland reserve, Victoria, Australia. Plos One **13**.

Mahmood et al (2018) evaluated the efficacy of glyphosate, pine oil, and mulching to control *Galenia pubescens* in an Australian degraded grassland. A year and a half (18 months) following application the glyphosate treatment and the pine oil treatment both reduced *Galenia* cover by about 50%.

Michalski, J., and Z. Cheng. 2018. Effects of "lights out" turfgrass renovation on plants, soil arthropod and nematode communities. Applied Soil Ecology **127**:144-154.

Michalski and Chang (2018) compared the efficacy of tarping with polypropylene and glyphosate applications to control Bermuda grass (*Cynodon dactylon*) in Hawaii. The tarping approach was more effective than glyphosate at keeping weeds away following the invader removals.

Ray, C. A., J. J. Sherman, A. L. Godinho, N. Hanson, and I. M. Parker. 2018. Impacts and Best Management Practices for Erect Veldtgrass (Ehrharta erecta). Invasive Plant Science and Management **11**:40-48.

Ray et al (2018) evaluated the efficacy of chemical and mechanical treatments for managing African veldt grass (Erharta erecta), and the non-target impacts on native plants. Twenty-two months following management treatments, veldt grass was reduced substantially with both mechanical and herbicide treatments, but herbicide application also produced greater reductions in native species cover and species richness.

Sartain, B. T., and C. R. Mudge. 2018. Effect of Winter Herbicide Applications on Bald Cypress (Taxachum distichum) and Giant Salvinia (Salvinia molesta). Invasive Plant Science and Management **11**:136-142.

Sartain and Mudge (2018) evaluated the efficacy of applying glyphosate in December, January, and February to treat the giant aquatic fern, Salvinia (*Salvinia molesta*). It is difficult to treat *Salvinia* other times of the year without harming the bald cypresss trees that it grows beneath. December applications of glyphosate reduced *Salvinia* cover by > than 80%, January applications reduced *Salvinia* cover by 60%-X, and February applications recued cover by 80%-100%.

Stover, H. J., M. A. Naeth, and S. R. Wilkinson. 2018. Transplanting Following Non-Native Plant Control in Rocky Mountain Foothills Fescue Grassland Restoration. Ecological Restoration **36**:19-27.

Stover et al (2018) compared the success of native forb, grass and shrubs that were planted into grassland restorations sites following invasive species control. Invasive plants were removed by steaming, cutting or, applying the recommended rate of glyphosate. Two years following planting forbs, grasses, and shrubs had higher survival where glyphosate had been used to remove invasive species.

Willoughby, I. H., J. Forster, and V. J. Stokes. 2018. Gaultheria shallon can be controlled by the herbicides picloram, triclopyr or glyphosate if they are applied at the correct time of year. New Forests **49**:757-774.

Willoubghy er al (2018) evaluated the efficacy of glyphosate, triclopyr, and picloram applications to control salal (Gaultheria shallon) in the UK where it limits forest regeneration. Overall glyphosate was the least effective treatment. A single application only reduced salal cover by less than 30% and after 4 application salal still had 30-40% cover.

ENVIRONMENTAL FATE

Aslam, S., A. Iqbal, F. Lafolie, S. Recous, P. Benoit, and P. Garnier. 2018. Mulch of plant residues at the soil surface impact the leaching and persistence of pesticides: A modelling study from soil columns. Journal of Contaminant Hydrology **214**:54-64.

Aslam et al. (2018) evaluated the potential for glyphosate residues on crop plants that had been chopped up at used as mulch to be washed off and leach into the soil. Glyphosate was applied that a rate of 0.24 g/L to cut up pieces of maize and dolichos plants an artificial rain treatment that mimicked either high and infrequent intensities or light and frequent intensities. The rain treatments were applied 2 hours after the glyphosate was applied. One day after the rain treatments were applied 45% of the glyphosate had been washed into the soil. At MROSD glyphosate is not applied to dead plant tissue.

Bradley, P. M., C. A. Journey, K. M. Romanok, L. B. Barber, H. T. Buxton, W. T. Foreman, E. T. Furlong, S. T. Glassmeyer, M. L. Hladik, L. R. Iwanowicz, D. K. Jones, D. W. Kolpin, K. M. Kuivila, K. A. Loftin, M. A. Mills, M. T. Meyer, J. L. Orlando, T. J. Reilly, K. L. Smalling, and D. L. Villeneuve. 2017. Expanded Target-Chemical Analysis Reveals Extensive Mixed Organic-Contaminant Exposure in US Streams. Environmental Science & Technology **51**:4792-4802.

Bradley et al. (2017) collected water samples from 38 streams across the country (USA). Glyphosate (or its metabolite AMPA) were present in 79% of the samples, caffeine and the diabetes drug metformin were present in 74% and 66% of the samples, respectively. The concentrations were not described so it is difficult to determine the importance of these findings

Cassigneul, A., P. Benoit, V. Bergheaud, V. Dumeny, V. Etievant, Y. Goubard, A. Maylin, E. Justes, and L. Alletto. 2016. Fate of glyphosate and degradates in cover crop residues and underlying soil: A laboratory study. Science of the Total Environment **545**:582-590.

Grandcoin, A., S. Piel, and E. Baures. 2017. AminoMethylPhosphonic acid (AMPA) in natural waters: Its sources, behavior and environmental fate. Water Research **117**:187-197.

Grandcoin et al. (2017) is an excellent review paper that describes in detail glyphosate and AMPA transport and persistence in the environement. One important conclusion they draw in this review sithat glyphosate and AMPA foundin the environmet are primarily from agricultural runoff and that urban inputs are negligable.

Louch, J., V. Tatum, G. Allen, V. C. Hale, J. McDonnell, R. J. Danehy, and G. Ice. 2017. Potential Risks to Freshwater Aquatic Organisms Following a Silvicultural Application of Herbicides in Oregon's Coast Range. Integrated Environmental Assessment and Management **13**:396-409.

Louch et al. (2017) study the persistence of herbicides that were applied by helicopter onto a forest plantation site in Oregon. Two of the herbicides include in the tankmixture were glyphosate and imazapyr. Water samples were collected from a stream that passed through the plantation site. Imazapyr was not detectd in the water samples. Glyphosate was detected in water samples collected during the application and the concentraion increased post application following a strom event but this glyphosate pulse dispated within 12 hours . Findings from this study suggest that aqautic organisms are exposed to multiple short term pulse of glyphosate following arieal applications to forestry sites.

Nguyen, N. K., U. Doerfler, G. Welzl, J. C. Munch, R. Schroll, and M. Suhadolc. 2018. Large variation in glyphosate mineralization in 21 different agricultural soils explained by soil properties. Science of the Total Environment **627**:544-552.

In a lab experiment, Nguyen et al. (2018) evaluated the mineralization rates of glyphosate in 21 different agricultural soils that widley variaable soil propertied (e.g. texture, organic mater content, pH, cation exchange capacity). ^{14}C labled glyphosate was added to the soil and glyphosate mineralization was measured for 32 days. Glyphosate mineralization varied from 7 to 70% of the amount initially applied adndepend on soil properties. Glyphosate mineralization started immediately after application, the highest mineralization rates were observed within the first 4 days in most of the 21 soils.

Ines Bonansea, R., I. Filippi, D. Alberto Wunderlin, D. J. Gabriel Marino, and M. Valeria Ame. 2018. The Fate of Glyphosate and AMPA in a Freshwater Endorheic Basin: An Ecotoxicological Risk Assessment. Toxics **6**.

Ines Bonansea et al. (2018) measured glyposate in water and sediment in a river that crosses through intensive agricultual ares. Sample were colleced in agricultural and non agricultural stretches of the river. Glyphosate (or its metabolite, AMPA) was found in much higher concentrations in both the water and sediment in the site that was near to the agricultural area. The concentrations of glyphosate in sedament was 20% higher than the water. Even in theagricutural area the concentration did not exceed the regulartory limits.

Poiger et al. (2017) detected glyphosate or AMPA in the majority of surface water samples collected near Zurich Switzerland. The samples had a median glyphosate concentration of 0.11-.20 ug/L. They concluded that non-agricultural uses can contribute significantly to glyphosate contamination in surface water.

Richards, B. K., S. Pacenka, M. T. Meyer, J. E. Dietze, A. L. Schatz, K. Teuffer, L. Aristilde, and T. S. Steenhuis. 2018. Antecedent and Post-Application Rain Events Trigger Glyphosate Transport from Runoff-Prone Soils. Environmental Science & Technology Letters **5**:249-254.

Richardson et al. (2018) measured glyphosate concentraions in outflow after broadcast spraying glyhosate onto a perrienial grassland field. Rainfall triggered the outflows and occurred 3-13 days affet the herbicide applications. Glyphosate concentrations ranged from 0-9- ug/L andwere highest in the outflow following the first rain. The amount of glyphosate in the outflow was primarily driven by the wetness of the soil at the time of the application. To avoid moibiization of glyphosate avoid spraying when the soils are wet.

Sviridov, A. V., T. V. Shushkova, I. T. Ermakova, E. V. Ivanova, D. O. Epiktetov, and A. A. Leontievsky. 2015. Microbial Degradation of Glyphosate Herbicides (Review). Applied Biochemistry and Microbiology **51**:188-195.

1.2 IMAZAPYR

HUMAN HEALTH RISKS

No studies were published between 2015 and 2018 that investigated human health risks associated with imazapyr.

ECOLOGICAL RISKS

Babalola, O. O., and J. H. Van Wyk. 2018. Comparative Early Life Stage Toxicity of the African Clawed Frog, Xenopus laevis Following Exposure to Selected Herbicide Formulations Applied to Eradicate Alien Plants in South Africa. Archives of Environmental Contamination and Toxicology **75**:8-16.

Babalola et al. (2018) examined the toxicity of glyphosate (Roundup) and amazapyr on embryos, premetamorphic larve and prometamorphic larvae of African clawed frogs (*Xenopus laevis*). They determined that the Roundup LC50s for the frog embryos, premetamorphic larve and prometamorphic was 1.05, 0.89, and 2.75 mg/L, respectivley. For imazapyr the LC50s for embryos, premetamorphic larve and prometamorphic was 36, 32.8, and 173.5 mg/L, respectively. Findings from this study suggest that young tadpoles are more sensitive to herbicides than embryos or protometamorphic tadpoles.

Enloe, S. F., and M. D. Netherland. 2017. Evaluation of three grass-specific herbicides on torpedograss (Panicum repens) and seven nontarget, native aquatic plants. Journal of Aquatic Plant Management **55**:65-70.

Enloe and Netherland (2017) compared the effects of clethodim, a grass specific herbicide, with non-selective herbicides; glyphosate and imazapyr on target invasive grasses and non-target native aquatic forbs. Imazapyr and glyphosate reduced nongrass biomass by 64-100%, whereas clothdium did not affect native forbs at all. Clothdim, glyphoste and imazapyr all reduced the target grass cover by 69-85%.

Golombieski, J. I., F. J. Sutili, J. Salbego, D. Seben, L. T. Gressler, J. A. da Cunha, L. T. Gressler, R. Zanella, R. D. Vaucher, E. Marchesan, and B. Baldisserotto. 2016. Imazapyr plus imazapic herbicide determines acute toxicity in silver catfish *Rhamdia quelen*. Ecotoxicology and Environmental Safety **128**:91-99.

Golombieski et al. (2016) evaluated the acute toxicity of an herbicide formulation that contains both imazapyr and imazapic on the fish (*Rhamdia quelen*). Fish were exposed to 0, 0.488, or 4.88 μg/L of the herbicide and hematological, biochemical, immunological, ionoregulatory and enzymatic functions were measured. . No hematological measurements were affected by exposure to the low (0.488 μg/L) concentration of the herbicide. Of the 11 hematological tests conducted only 2 were affected by 96 hours of exposure of herbicide at the higher concentration (4.88 μg/L). Of the 13 biochemical tests performed only cortisol was affected by 96 hours of exposure to the highest concentration. Hemolysis declined in response to the high herbicide concentration. The low concentration resulted in lowered bactericidal activity, but the higher concentration did not. The lack of effect at the higher concentration makes interpretation of these herbicide effect on immunological responses difficult. Potassium increase, sodium decreased and chloride remained unchanged following exposure to the high herbicide concentration and the low dose had no effect on these ions. ADP was also not affected by the herbicide at the low or high concentrations following 96 hours of exposure. These findings taken together suggest that the herbicide formulation puts some stress on fish but that overall fish health is minimally impacted by 96 hours of acute exposure. From this study it is not possible to determine how imazapyr alone could affect fish so the findings have little relevance to MROSD where imazapyr is applied by itself.

Louch, J., V. Tatum, G. Allen, V. C. Hale, J. McDonnell, R. J. Danehy, and G. Ice. 2017. Potential Risks to Freshwater Aquatic Organisms Following a Silvicultural Application of Herbicides in Oregon's Coast Range. Integrated Environmental Assessment and Management **13**:396-409.

Louch et al. (2017) study the persistence of herbicides that were applied by helicopter onto a forest plantation site in Oregon. Two of the herbicides include in the tankmixture were glyphosate and imazapyr. Water samples were collected from a stream that passed through the plantation site. Imazapyr was not detectd in the water samples. Glyphosate was detected in water samples collected during the application and the concentraion increased post application following a strom event but this glyphosate pulse dispated within 12 hours . Findings from this study suggest that aqautic organisms are exposed to multiple short term pulse of glyphosate following arieal applications to forestry sites.

ENVIRONMENTAL FATE

Atitar, M. F., A. Bouziani, R. Dillert, M. El Azzouzi, and D. W. Bahnemann. 2018. Photocatalytic degradation of the herbicide imazapyr: do the initial degradation rates correlate with the adsorption kinetics and isotherms? Catalysis Science & Technology **8**:985-995.

Atitar et al. (2018) evaluated the role of pH and light in the degration of imazapyr suspended in water. They found that the maximum amount of imazapyr is adsorbed at a pH of 3 and that as pH increases imazapyr adsorption decreases. They further found that the rate of imazapyr adsorption is higher in the light than in the dark, but that the effect that light has on adsorbtion is dependent upon pH.

Bundt, A. C., L. A. Avila, A. Pivetta, D. Agostinetto, D. P. Dick, and P. Burauel. 2015. Imidazolinone degradation in soil in response to application history. Planta Daninha **33**:341-349.

Bundt et al. (2015) compared the degradation rates of imazapyr in soils with and without previous exposure to herbicide formulations that contained imazapyr. The degradation rate was determined by CO_2 emissions from the soil. Prior exposure to imazapyr containing herbicides did not increase the rate of microbial degradation of imazapyr following subsequent applications.

Douglass, C. H., S. J. Nissen, and A. R. Kniss. 2016. Efficacy and environmental fate of imazapyr from directed helicopter applications targeting Tamarix species infestations in Colorado. Pest Management Science **72**:379-387.

Douglass et al. (2016) evaluated the imazapyr residues in soil under and near to aerial sprayed Tamarisk stands. They determined that 71% of the imazapyr was intercepted by the target plant, which means that 29% was deposited into the surrounding environment. They further determined that imazapyr in the soil under the Tamarisk canopy was less than outside the canopy, but the degradation rates were 4 times faster outside the canopy. Outside the canopy >99% of the imazapyr degraded within 6 months, whereas under the canopy it took 15 months to degrade 99% of the imazapyr .

Ozcan, C., U. K. Cebi, M. A. Gurbuz, and S. Ozer. 2017. Residue Analysis and Determination of IMI Herbicides in Sunflower and Soil by GC-MS. Chromatographia **80**:941-950.

Ozcan et al. (2017) measured imazapyr residues in sunflower fields where imazapyr is intensivly used to control weeds. The did not detect imazypr in the soils from 0-60 cm deep.

Porfiri, C., J. C. Montoya, W. C. Koskinen, and M. P. Azcarate. 2015. Adsorption and transport of imazapyr through intact soil columns taken from two soils under two tillage systems. Geoderma **251**:1-9.

Porfiti et al. (2015) confirmed that imazapyr wakly adsorbs to soil, but that soils with higher clay content, and therefore higeer cation exchange capacity can adsob more imazapyr.

Singh, B., and K. Singh. 2016. Microbial degradation of herbicides. Critical Reviews in Microbiology **42**:245-261.

Singh and Sing (2016) is a review paper that descibes the mode of action and the degredation of imazapyr and other herbicides.

EFFICACY

Boyd, N. S., S. N. White, and T. Larsen. 2017. Sequential Aminopyralid and Imazapyr Applications for Japanese Knotweed (Fallopia japonica) Management. Invasive Plant Science and Management **10**:277-283.

Copied from the management implication statement in manuscript: Japanese knotweed is a common invasive plant species that occurs along waterways, in parks, in abandoned agricultural fields, and in other disturbed areas. An established stand of knotweed outcompetes

other vegetation and reduces localized species diversity. It also destabilizes the banks of waterways and can make them more susceptible to erosion. A variety of management techniques have been evaluated, but herbicides are consistently the
most cost-effective approach. Boyd et al 2018 found that an application of aminopyralid at 120 g/ ha when Japanese knotweed shoots are approximately 30-cm tall did not adequately control knotweed but did suppress shoot growth. Imazapyr applications at 720 g/ha was much more effective when applied any time between maximum height and just before shoot senescence in the fall. By 52 weeks after treatment, all imazapyr application timings provided similar levels of control, with multiple imazapyr applications providing no additional benefit. In large stands, early aminopyralid applications may suppress knotweed growth and facilitate late season imazapyr applications. This technique is not recommended along waterways, as it kills most vegetation, leaving areas of bare soil that would be susceptible to erosion.

Cole, E., A. Lindsay, M. Newton, and J. D. Bailey. 2018. Vegetation Control and Soil Moisture Depletion Related to Herbicide Treatments on Forest Plantations in Northeastern Oregon. Weed Technology **32**:461-474.

Cole et al. (2018) evaluated the effectiveness of spring and summer applications of several herbicides (alone or in combination) in reducing the abundance of plants competing with planted forestry conifer seedlings in the Pacific Northwest. The spring herbicide treatments included sulfometuron, sulfometuron + glyphosate, sulfometuron + clopyralid, atrazine, atrazine + imazapyr, imazapyr, and hexazinone. Summer treatments included: glyphosate + imazapyr, aminopyralid + sulfometuron, and glyphosate + atrazine, imazapyr, and glyphosate. Results varied by site, year, and season of application. In general, sulfometuron (0.14 kg/ ha) alone and in various mixtures, imazapyr (0.42 kg/ha), and hexazinone (1.68 kg/ha) resulted in 3 to 17% cover of forbs and grasses in the first-year when applied in spring. Sulfometuron + glyphosate (2.2 kg/ha) consistently reduced grasses and forbs for the first year when applied in summer, but forbs recovered in the second year on two of three sites. Aminopyralid (0.12 kg/ha) + sulfometuron applied in summer also led to comparable control of forb cover. In the second year after treatment, forb cover in treated plots was similar to levels in nontreated plots, and some species of forbs had increased relative to nontreated plots. Imazapyr (0.21 and 0.42 kg/ha) at either rate, in either spring or summer, or at lower rate (0.14 kg ha–1) with glyphosate in summer, provided the best control of shrubs. Total vegetative cover was similar across all treatments seven and eight years after application, and differences in vegetation were related to site rather than treatment.

Farthing et al (2018) evaluated the efficacy of glyphosate and imazapyr at removing Bermudagrass (*Cynodon dactylon*) from grasslands in Texas. Glyphosate applied once reduced Bermudagrass cover by 70%. Two applications of glyphosate reduced bermudagrass cover by >95%. Glyphosate alone was as effective as mowing prior to glyphosate application. Imazapyr applied only once was as effective at controlling Bermudagrass as multiple applications of glyphosate. However, imazapyr's half-life in soil ranges from one to five months and non-target species may be adversely affected.

Isbister, K. M., E. G. Lamb, and K. J. Stewart. 2017. Herbicide Toxicity Testing with Non-Target Boreal Plants: The Sensitivity of *Achillea millefolium* and *Chamerion angustifolium* to Triclopyr and Imazapyr. Environmental Management **60**:136-156.

Isbister et al. (2017) evaluated the sensitivity of yarrow (*Achillea millefolium*) and fireweed (*Chamerion angustifolium*), to imazapyr and triclopyr. These plants are non-target plants that frequently co-occur with target weeds under powerlines. Triclopyr caused extensive damage to yarrow at <50% of the maximum field application rate and was lethal to fireweed at the lowest dose tested. Both species were extremely to imazapyr and the lowest dose tested cause >75% mortality

Knezevic, S. Z., O. A. Osipitan, M. C. Oliveira, and J. E. Scott. 2018. Lythrum salicaria (Purple Loosestrife) Control with Herbicides: Multiyear Applications. Invasive Plant Science and Management **11**:143-154.

Knezevic et al. (2018) evaluated the effectiveness of 14 herbicide treatments for purple loosestrife (*Lythrum salicaria*) control over a 10-year period. Here I will describe only the treatments relevant to MROSD. Glyphosate was applied at 2.2 and 3.4 kg/ha, triclopyr at 1.3 and 2.1 kg/ha, and imazapyr at 1.1 and 1.7 kg/ha. The herbicides were applied every year until 100% control was achieved. The high application rates of glyphosate and imazapyr provided excellent purple loosestrife control (≥90%) that lasted at least a year following application, and by year 3 both herbicides had eradicated the invader. It took 9 years to eradicate purple loosestrife with triclopyr. The older the purple loosestrife, the more years of follow-up application were needed. Generally, there were higher percentages of grasses following triclopyr applications and higher cover of broadleaf species following glyphosate or imazapyr treatments.

1.3 *AMINOPYRLID*

HUMAN HEALTH RISKS

Settivari, R. S., S. C. Gehen, R. A. Amado, N. R. Visconti, D. R. Boverhof, and E. W. Carney. 2015. Application of the KeratinoSens (TM) assay for assessing the skin sensitization potential of agrochemical active ingredients and formulations. Regulatory Toxicology and Pharmacology **72**:350-360.

Researches at Dow Chemical, the manufacturers of Milestone, evaluated a new method to test for skin sensitivity caused by pesticides, and they trademarked the method as KeratinoSens™. With this new method Settivari et al. (2015) confirmed that aminopyralid does not cause skin sensitivity

ECOLOGICAL RISKS

PLANTS

DiTomaso, J. M., and G. B. Kyser. 2015. Effects of Aminopyralid on California Annual Grassland Plant Communities. Invasive Plant Science and Management **8**:98-109.

DiTomaso and Kyser (2015) measured the non-target effects of 53 and 123 g/ha of aminopyralid on individual species cover and species richness over three growing seasons in two California grassland locations. Aminopyralid was used to treat yellow star thistle. Treated plots were compared to untreated plots. In the first season after treatment, both rates of aminopyralid reduced dicot cover significantly, particularly members of the Asteraceae and Fabaceae. Treated plots also showed reduced species richness. However, these differences were less pronounced in the second season after treatment, particularly at the low rate. By the third season after treatment in both sites, there were no longer any significant effects on cover or species richness at the low herbicide rate. On California annual grasslands, winter applications of low rates of aminopyralid have been shown to give excellent control of yellow starthistle, providing long-term benefits to grassland ecosystems. Results from this study suggest that negative impacts of aminopyralid on the desirable native forb community are transitory.

Goodman, L. E., A. F. Cibils, R. L. Steiner, J. D. Graham, and K. C. McDaniel. 2015. Control of Silky Crazyweed (Oxytropis sericea) with Aminopyralid+2,4-D and Picloram+2,4-D on Native Rangeland. Invasive Plant Science and Management **8**:401-408.

Along with comparing the efficacy of aminopyralid + 2,4-D and picloram + 2,4-D for controlling silky crazyweed (*Oxytropis sericea; in the Fabaceae*), Goodman et al (2015), also compared effects on non-target grass and forbs cover. Grass cover was approximately 11% higher for both herbicide treatments compared to the control. Fifteen months after treatment controls had the greatest forb cover followed by the picloram + 2,4-D– treatments, and the aminopyralid + 2,4-

D–treated plots had lower forb cover percentages than the picloram + 2,4- D plots did (Table 4). This suggests that aminopyralid + 2,4-D may be less selective than picloram + 2,4-D.

Jorgensen, N. A., M. J. Moechnig, M. B. Halstvedt, and M. J. Renz. 2017. Native Forb Establishment following Application of Aminopyralid or Clopyralid. Invasive Plant Science and Management **10**:90-98.

Jorgensen et al. (2017) evaluated the effects aminopyralid and clopyralid on seeded non-target common forb species. They applied aminopyralid (54 or 123 g/ ha), clopyralid (237 and 420 g/ ha), or aminopyralid + clopyralid (54 + 237 g/ ha) in July and then seeded the treatment areas the following fall and spring. The herbicide treatments did not affect seedling establishment. Results suggest native forbs can tolerate these herbicides when applied at least 4 months prior to seeding.

Lym, R. G., R. L. Becker, M. J. Moechnig, M. B. Halstvedt, and V. F. Peterson. 2017. Native Grass Establishment following Application of Pyridine Herbicides. Invasive Plant Science and Management **10**:110-117.

Lym et al. (2017) evaluated the effects of aminopyralid, clopyralid, or picloram applied in the fall to areas seeded the following fall or spring with native cool and warm season grasses. The herbicides were applied at standard rates. Aminopyralid did not reduce seedling establishment in either fall or spring seeding. For the most part none of the herbicide applications affected seedling establishment of most of the seeded grasses. The exceptions were a slight reduction in fall-seeded wheatgrass in the clopyralid and picloram treatments and a Canadian wild rye in the picloram treatment.

McManamen, C., C. R. Nelson, and V. Wagner. 2018. Timing of seeding after herbicide application influences rates of germination and seedling biomass of native plants used for grassland restoration. Restoration Ecology **26**:1137-1148.

McManamen et al. (2018) conducted greenhouse and field experiments to evaluate the impacts of herbicides on native seed additions. They also assessed the timing between herbicide application and seeding to determine how to optimize restoration success. They compared two herbicides, picloram and aminopyralid, and applied each at the recommended rates (0.52 L/ha for aminopyralid and 4.78 L/ha for picloram). They then seeded in 10 native plants commonly used in grassland restoration at 0, 3,6, 9 or 11 months following the herbicide treatments. In the greenhouse, herbicides negatively impacted germination of four species over the entire 11-month trail whereas six showed less sensitivity over time. In the field study, germination rates and biomass were lower in herbicide-treated plots sprayed in the spring than those in control plots for 75% of seeded species. The field plots sprayed in the fall herbicides only decreased germination and biomass for 25% of seeded species.

SOIL MICROBIAL COMMUNITY

Tomco, P. L., K. N. Duddleston, E. J. Schultz, B. Hagedorn, T. J. Stevenson, and S. S. Seefeldt. 2016. Field degradation of aminopyralid and clopyralid and microbial community response to application in Alaskan soils. Environmental Toxicology and Chemistry **35**:485-493.

Due to the cold temperatures at high latitudes there is concern that aminopyralid and clopyralid may persist longer in soil than in warmer regions where degradation occurs rapidly. Tomco et al. (2016) sprayed plowed agricultural fields with aminopyralid or clopyralid at the recommended rates. Both herbicides degraded rapidly over the first 29-period following the herbicide applications. After 90 days, residual clopyralid was <0.02μg/g at one field site and 0.046 μg/g at the other. Aminopyralid was slightly more persistent with 0.049 μg/g at one site and 0.12 μg/g at the other site. No differences in microbial diversity or community composition was found in treated and untreated soil the herbicides.

ENVIRONMENTAL FATE

Karise, R., R. Raimets, V. Bartkevics, I. Pugajeva, P. Pihlik, I. Keres, I. H. Williams, H. Viinalass, and M. Mand. 2017. Are pesticide residues in honey related to oilseed rape treatments? Chemosphere **188**:389-396.

Karise et al (2017) collected honey from hives that were in the general vicinity of oil seed crops. The honey was analyzed for 47 pesticide residues, includiing glyphosate, aminopyralid and clopyralid. Aminopyralid was not detected in the honey. Clopyralid was found in 64% of the samples, and two samples were above the maximum residue limit. Glyphosate was found in 21% of the samples with two samples were above the maximum residue limit.

Li, W., J. Mao, X. Dai, X. Zhao, C. Qiao, X. Zhang, and E. Pu. 2018. Residue determination of triclopyr and aminopyralid in pastures and soil by gas chromatography-electron capture detector: Dissipation pattern under open field conditions. Ecotoxicology and Environmental Safety **155**:17-25.

Li et al. (2018) used a new method to evaluate the of triclopyr and aminopyralid residues in forage grass, hay, and soil and found that over time triclopyr and aminopyralid dissipated. In forage grass, both compounds degraded rapidly over the first 14- or 21-d period and at a slow rate over the remainder of experimental days. In soil, they degraded at a relatively slow rate, and dissipated steadily to below or close to the LOQ by 60-d post application. The half-lives of triclopyr were 1.4–1.8 d and 6.2–9.0 d and aminopyralid were 1.7–2.1 d and 8.2–10.6 d in terms of forage grass and soil, respectively. The residues concentrations 7 days following the treatments, the residues of aminopyralid and triclopyr in forage grass and hay were lower than maximum limits allowed by the EPA (USA).

EFFICACY

Boyd, N. S., S. N. White, and T. Larsen. 2017. Sequential Aminopyralid and Imazapyr Applications for Japanese Knotweed (Fallopia japonica) Management. Invasive Plant Science and Management **10**:277-283.

Copied from the management implication statement in manuscript: Japanese knotweed is a common invasive plant species that occurs along waterways, in parks, in abandoned agricultural fields, and in other disturbed areas. An established stand of knotweed outcompetes other vegetation and reduces localized species diversity. It also destabilizes the banks of waterways and can make them more susceptible to erosion. A variety of management techniques have been evaluated, but herbicides are consistently the most cost-effective approach. Boyd et al 2018 found that an application of aminopyralid at 120 g/ ha when Japanese knotweed shoots are approximately 30-cm tall did not adequately control knotweed but did suppress shoot growth. Imazapyr applications at 720 g/ha was much more effective when applied any time between maximum height and just before shoot senescence in the fall. By 52 weeks after treatment, all imazapyr application timings provided similar levels of control, with multiple imazapyr applications providing no additional benefit. In large stands, early aminopyralid applications may suppress knotweed growth and facilitate late season imazapyr applications. This technique is not recommended along waterways, as it kills most vegetation, leaving areas of bare soil that would be susceptible to erosion.

Carter, T. R., and R. G. Lym. 2017. Canada Thistle (Cirsium arvense) Affects Herbage Production in the Northern Great Plains. Invasive Plant Science and Management **10**:332-339.

Copied from the management implication statement in manuscript: Canada thistle infestations in crops often cause at least a 50% reduction in yield, and the losses have been assumed to be similar in non-cropland. However, reports on the loss of production from Canada thistle in pasture, rangelands, and wildlands have been inconsistent. In a two-part study, the change in yield of grasses, forbs, and woody plants following Canada thistle control with aminopyralid was evaluated in two prairie sites, while production was also compared in infested versus non-infested sites within wildlife management areas in North Dakota at 20 sites. In general, grass, broadleaf, woody, and total plant yield were similar regardless of the near-complete control of Canada thistle following aminopyralid application. Grass yield increased 365 kg/ ha the year after treatment at one location, with no change in forb or woody species production. Similarly, there was minimal or no loss of production from Canada thistle in the wildlife management areas located in two separate Major Land Resource Areas (MLRAs) in east-central and central North Dakota. The only exception was an increase in grass production of 425 kg/ ha at one of the MLRAs, with no change in broadleaf or woody species production in the Canada thistle infested compared with the non-infested sites. Canada thistle does not appear to consistently reduce production of other species in non-cropland. However, Canada thistle control in these areas is still desirable, even if not cost-effective, for the increased hay quality, livestock health benefits, and improved habitat for native species.

Cole, E., A. Lindsay, M. Newton, and J. D. Bailey. 2018. Vegetation Control and Soil Moisture Depletion Related to Herbicide Treatments on Forest Plantations in Northeastern Oregon. Weed Technology **32**:461-474.

Cole et al. (2018) evaluated the effectiveness of spring and summer applications of several herbicides (alone or in combination) in reducing the abundance of plants competing with planted forestry conifer seedlings in the Pacific Northwest. The spring herbicide treatments included sulfometuron, sulfometuron + glyphosate, sulfometuron + clopyralid, atrazine, atrazine + imazapyr, imazapyr, and hexazinone. Summer treatments included: glyphosate + imazapyr, aminopyralid + sulfometuron, and glyphosate + atrazine, imazapyr, and glyphosate. Results varied by site, year, and season of application. In general, sulfometuron (0.14 kg/ ha) alone and in various mixtures, imazapyr (0.42 kg/ha), and hexazinone (1.68 kg/ha) resulted in 3 to 17% cover of forbs and grasses in the first-year when applied in spring. Sulfometuron + glyphosate (2.2 kg/ha) consistently reduced grasses and forbs for the first year when applied in summer, but forbs recovered in the second year on two of three sites. Aminopyralid (0.12 kg/ha) + sulfometuron applied in summer also led to comparable control of forb cover. In the second year after treatment, forb cover in treated plots was similar to levels in nontreated plots, and some species of forbs had increased relative to nontreated plots. Imazapyr (0.21 and 0.42 kg/ha) at either rate, in either spring or summer, or at lower rate (0.14 kg ha–1) with glyphosate in summer, provided the best control of shrubs. Total vegetative cover was similar across all treatments seven and eight years after application, and differences in vegetation were related to site rather than treatment.

DiTomaso, J. M., G. B. Kyser, D. J. Lewis, and J. A. Roncoroni. 2017. Conventional and Organic Options for the Control of Woolly Distaff Thistle (Carthamus lanatus). Invasive Plant Science and Management **10**:72-79.

DiTomaso and et al (2017) conducted two field experiments to evaluate the efficacy of different control options for *Carthamus lanatus* (Woolly distaff thistle). *Carthamus lanatus* is a long-lived winter annual that occurs on in coastal counties in Northern California. They compared several conventional herbicides at two timings and rates. They also compared a conventional herbicide treatment with organic and organic control methods, including an organic herbicide (mixture of capric and caprylic acids). Results of the conventional herbicide treatments showed most spring applications (March or April) of aminopyralid, aminocyclopyrachlor, clopyralid, and combinations of aminopyralid + triclopyr, or aminocyclopyrachlor + chlorsulfuron had greater than 99% control of *Carthamus lanatus,* with fewer than 1.5 seedlings per 27-m2 plot by the end of the growing season. Higher rates were generally necessary to achieve the same level of control with winter (January) applications. In the organic herbicide treatments, the most consistent treatment was a combination of mowing followed by 9% (v/v) or the organic herbicide. This treatment was slightly less effective compared with aminopyralid but did have better than 95% control of *Carthamus lanatus*. The results of this study provide control options for both conventional and organic ranching practices where woolly *Carthamus lanatus* is a problem.

Enloe, S. F., N. J. Loewenstein, D. Streett, and D. K. Lauer. 2015. Herbicide Treatment and Application Method Influence Root Sprouting in Chinese Tallowtree (Triadica sebifera). Invasive Plant Science and Management **8**:160-168.

Enloe et al (2015) compared the effectiveness of 6 different herbicides in controlling Chinese tallowtree (*Triadica sebifera*). They used triclopyr ester and triclopyr amine, aminocyclopyrachlor, aminopyralid, fluroxypyr, and imazamox for foliar, cut stump, and basal bark treatments in natural areas and measured root sprouting response to herbicide treatments. Aminocyclopyrachlor, aminopyralid, fluroxypyr, and imazamox all controlled Chinese tallowtree similar to, or better than, triclopyr. Aminocyclopyrachlor resulted in greater mortality of Chinese tallowtree than triclopyr did in foliar and basal bark studies, whereas aminopyralid was more effective than triclopyr in cut stump studies. Fluroxypyr resulted in greater mortality than triclopyr in cutstump and basal bark studies but not in foliar studies. Aminopyralid reduced total sprouting better than all other treatments in the cut stump study.

Goodman, L. E., A. F. Cibils, R. L. Steiner, J. D. Graham, and K. C. McDaniel. 2015. Control of Silky Crazyweed (Oxytropis sericea) with Aminopyralid+2,4-D and Picloram+2,4-D on Native Rangeland. Invasive Plant Science and Management **8**:401-408.

Goodman et al. (2015) compared the efficacy of aminopyralid + 2,4-D and picloram + 2,4-D for controlling silky crazyweed (*Oxytropis sericea; in the Fabaceae*) for two growing seasons. picloram + 2,4-D was applied at a rate of 0.3 kg/ha picloram + 1.1 kg/ha of 2,4-D, and aminopyralid + 2,4-D was applied at a rate of 0.1 kg/ha aminopyralid + 1.2 kg/ha of 2,4-D. Aminopyralid + 2,4-D reduced crazyweed density by 95% 15 months after treatment. Whereas the picloram + 2,4-D treatment reduced density by 80%. These differences were not statistically different from each other, meaning that they worked equally as well. Grass cover was approximately 11% higher for both herbicide treatments compared to the control. Fifteen months after treatment controls had the greatest forb cover followed by the picloram + 2,4-D–treatments, and the aminopyralid + 2,4-D–treated plots had lower forb cover percentages than the picloram + 2,4- D plots did (Table 4). This suggests that aminopyralid + 2,4-D may be less selective than picloram + 2,4-D.Grass biomass remained similar within treatments over time for control, aminopyralid + 2,4-D and picloram +2,4-D plots, and was similar in all plots 15 moths after treatment.

Gramig, G. G., and A. C. Ganguli. 2015. Managing Canada Thistle (Cirsium arvense) in a Constructed Grassland with Aminopyralid and Prescribed Fire. Invasive Plant Science and Management **8**:243-249.

Gramig and Gangul (2015) evaluated the use of aminopyralid and aminopyralid followed by prescribed fire to control Canada thistle (*Cirsium arvense*). Aminopyralid effectively controlled Canada thistle 1- and 2-years post-treatment. Aminopyralid alone reduced Canada thistle density by 94-99.7% and combined with prescribed fire the following spring it reduced stem density by 84-98.8%.

Harrington, T. B., D. H. Peter, and R. A. Slesak. 2018. Logging debris and herbicide treatments improve growing conditions for planted Douglas-fir on a droughty forest site invaded by Scotch broom. Forest Ecology and Management **417**:31-39.

Harrington et al. (2018) evaluated the efficacy of controlling Scotch broom with slash debris left in place following logging and applications of aminopyralid alone and in combination with triclopyr ester. Scotch broom cover decreased from 20% to 0% as a result of the logging debris and herbicide treatments and as a result soil moisture increased which improved the survival and growth of planted Douglas-fir.

James, T. K., and C. A. Dowsett. 2015. Herbicide responses of mat-forming weeds of forest remnants in New Zealand. New Zealand Plant Protection **68**:1-6.

James and Dowsett (2015) compared the effectiveness of aminopyralid, triclopyr and aminopyralid + triclopyr in defoliating 6 different mat forming invasive plants (*Tradescantia fluminensis, Plectranthus ciliatus, Asparagus scandens, Hedera helix, Lamium galeobdolon* and *Selaginella kraussiana) that* limit native plant regeneration. They compared the speed, duration and extent of defoliation by the herbicides when applied at quarter, half and full label-recommended application rates for general weed control using a backpack sprayer. Low rates (338-675 g/ha) of triclopyr resulted in >90% defoliation of *T. fluminensis, A. scandens* and *L. galeobdolon*, but a higher rate of 1350 g/ha only gave 85% defoliation of *H. helix*. Aminopyralid was effective against *S. kraussiana* but was very slow acting.

Phillips, W. P., T. D. Israel, T. C. Mueller, G. R. Armel, D. R. West, J. D. Green, and G. N. Rhodes, Jr. 2016. Utility of Aminocyclopyrachlor for Control of Horsenettle (Solanum carolinense) and Tall Ironweed (Vernonia gigantea) in Cool-Season Grass Pastures. Weed Technology **30**:472-477.

Phillips et al. (2016) evaluated the effectiveness of aminocyclopyrachlor (49 and 98 g/ha) and aminopyralid (88 g/ha) to control ironweed (*Veronia gigantean*) and horse nettle (*Solanum carolinense*) in pasture grass fields. They sprayed early, moderate, and late emergent plants. One year after treatment horse nettle was controlled 74% with aminocyclopyrachlor plus 2,4-D applied late following emergence. By one-year post treatment, tall ironweed treated early or late following emergence was controlled by 93% by aminocyclopyrachlor. Similar control was achieved with aminopyralid applied late. Both aminocyclopyrachlor and aminopyralid were found to reduce horse nettle and tall ironweed biomass the following year.

Rinella, M. J., J. S. Davy, G. B. Kyser, F. E. Mashiri, S. E. Bellows, J. J. James, and V. F. Peterson. 2018. Timing Aminopyralid to Prevent Seed Production Controls Medusahead (Taeniatherum caput-medusae) and Increases Forage Grasses. Invasive Plant Science and Management **11**:61-68.

Rinella et al. (2018) applied aminopyralid to Medusahead (*Taeniatherum caput-medusae*) in California grasslands to determine how reducing seed production in the current growing season influenced cover in the subsequent growing season. Aminopyralid was applied at 55, 123, and 245 g/ha in spring. The two higher rates were also applied in the fall to treat pre-emergent Medusahead seed. When applied in spring the low dose of 55 g/ha aminopyralid greatly limited seed production and subsequently reduced Medusahead cover to nearly zero. Fall aminopyralid applications were much less effective, even at the high application rate of 245 g/ha. The growing season after application, both spring and fall treatments tended to increase forage grasses, though spring treatments generally caused larger increases. In the discussion of these findings Rinella et al (2018) point out that yellow starthistle often co-occurs with Medusahead, but that starthistle is only in a basal rosette stage during spring (the optimal time to treat medusahead) and one application to treat both problems is not likely to work.

Spring, J. F., M. E. Thorne, I. C. Burke, and D. J. Lyon. 2018. Rush Skeletonweed (Chondrilla juncea) Control in Pacific Northwest Winter Wheat. Weed Technology **32**:360-363.

Spring et al. (2018) compared the efficacy of aminopyralid, clopyralid, aminocyclopyrachlor, dicamba, and 2,4-D of rush skeletonweed (*Chondrilla juncea*) control. Herbicides applied in spring and fall were compared. Clopyralid and aminopyralid provided good to excellent control of rush skeletonweed, and spring and fall applications reduced cover equally well.

Tran, H., K. Harrington, A. Robertson, and M. Watt. 2016. Assessment of herbicides for selectively controlling broom (Cytisus scoparius) growing with radiata pine (Pinus radiata) in New Zealand. New Zealand Journal of Forestry Science **46**.

Tran et al. 2017 evaluated several herbicide mixtures for use in controlling Scotch broom (*Cytisus scoparius*) in pine plantations. They applied 18 different herbicide treatments. The only herbicide treatments relevant to MROSD in this study were a low (half the recommended rate), medium (recommended rate) and high (2x recommended rate) applications of a clopyralid and aminopyralid mixture. The clopyralid /aminopyralid combinations gave good control of broom at all rates assessed. The highest rate of the clopyralid/triclopyr mix gave slightly better control than the medium rate.

1.4 CLOPYRALID

HUMAN HEALTH RISKS

Arena, M., D. Auteri, S. Barmaz, A. Brancato, D. Brocca, L. Bura, L. C. Cabrera, A. Chiusolo, C. Civitella, D. C. Marques, F. Crivellente, L. Ctverackova, C. De Lentdecker, M. Egsmose, Z. Erdos, G. Fait, L. Ferreira, L. Greco, A. Ippolito, F. Istace, S. Jarrah, D. Kardassi, R. Leuschner, A. Lostia, C. Lythgo, J. O. Magrans, P. Medina, D. Mineo, I. Miron, T. Molnar, L. Padovani, J. M. P. Morte, R. Pedersen, H. Reich, A. Sacchi, M. Santos, R. Serafimova, R. Sharp, A. Stanek, F. Streissl, J. Sturma, C. Szentes, J. Tarazona, A. Terron, A. Theobald, B. Vagenende, J. Van Dijk, L. Villamar-Bouza, and Efsa. 2018. Peer review of the pesticide risk assessment of the active substance clopyralid. Efsa Journal **16**.

This is a risk assessment carried out by the European Food and Safety Authority. They evaluated the risks associated with the use of clopyralid on winter cereals and grassland. Although the information is meant for guiding specific agricultural use of clopyralid, some of the information is relevant to natural land managers as well. One important conclusion is that there are important data gaps regarding the risks of clopyralid to human health and ecotoxicological effects on non-target organisms and to the degradation of the pesticide in the environment

ECOLOGICAL RISKS

Weir, S. M., S. Y. Yu, A. Knox, L. G. Talent, J. M. Monks, and C. J. Salice. 2016. Acute toxicity and risk to lizards of rodenticides and herbicides commonly used in New Zealand. New Zealand Journal of Ecology **40**:342-350.

Weir et al. (2016) evaluated the toxicity of several herbicides and pesticides at varying concentrations on western fence lizards (*Sceloporus occidentalis*). The chemicals evaluated included 5 rodenticides: brodifacoum, coumatetralyl, pindone, diphacinone and cholecalciferol, and five herbicides: glyphosate, clopyralid, triclopyr, metsulfuron-methyl and haloxyfop-methyl. Here I only describe the results of chemicals included in the MROSD invasive species management plan. Pesticide risk was determined by comparing the 96-hour LD50s to oral exposure models, and when exposure exceeds toxicity, this represents risk. The LD50s was > 1750 μg/g for glyphosate and clopyralid, 550 for triclopyr, and > 1750 for Cholecalciferol. It is important to note that the lizard toxicity value associated with triclopyr is still quite high (LD50 = 550 μg/g) and is probably environmentally unrealistic under normal application scenarios.

Marileo, L. G., M. A. Jorquera, M. Hernandez, G. Briceno, M. D. Mora, R. Demanet, and G. Palma. 2016. Changes in bacterial communities by post-emergent herbicides in an Andisol fertilized with urea as revealed by DGGE. Applied Soil Ecology **101**:141-151.

In a mesocosm experiment, Marileo et al. (2016) studied the interacting effects of urea and post emergent herbicides on bacterial communities. They evaluated 6 herbicides (MCPA, flumetsulam, fluroxypyr, triclopyr, clopyralid and picloram). Since urea or other fertilizers are not generally applied in natural land management I will only describe the effects of the relevant herbicides applied without urea. In the absence of urea, significant changes in total bacteria between the triclopyr-treated and control soils were observed, but only when the 2-fold-higher recommended dose of triclopyr was applied (1.94 mg/kg soil) at day 15. Changes in the ammonium oxidizing bacterial communities were also observed between the control soils and those that were treated with the 2-fold-higher recommended dose of triclopyr. Clopyralid did not change total bacteria or ammonium oxidizing bacterial communities. Residual triclopyr concentrations decreased by 81.4% 15 days following the application and clopyralid decreased by 73.7%.

Sura, S., M. J. Waiser, V. Tumber, R. Raina-Fulton, and A. J. Cessna. 2015. Effects of a herbicide mixture on primary and bacterial productivity in four prairie wetlands with varying salinities: An enclosure approach. Science of the Total Environment **512**:526-539.

Wetlands vary in their water quality parameters and this is reflected in productivity and aquatic plant community composition. Four wetlands within a natural area in Canada were studied to evaluate the impacts of herbicides on algal and bacterial microbial communities. The herbicide was a mixture of (2,4-D,MCPA, dicamba, clopyralid, bromoxynil, mecoprop, dichlorprop, and glyphosate). The concentration of each herbicide was applied at the recommended rate. Of the six herbicides of concern, only 2,4-D and MCPA were detected in the wetlands prior to herbicide treatment. Temperature, pH, specific conductivity, dissolved oxygen, alkalinity, dissolved organic carbon, nitrate, nitrite, total dissolved nitrogen, total dissolved phosphorus, total phosphorus, and particulate organic nitrogen, and carbon were found to be similar in wetland water samples collected from the control and treated areas in all four wetlands. The herbicide mixture increased primary productivity in the nutrient-sufficient wetland, and had no effect in the nutrient-deficient wetland. Since the six herbicides were combined in this study it is impossible to determine which herbicides contributed to changes in primary productivity and microbial communities.

Tomco, P. L., K. N. Duddleston, E. J. Schultz, B. Hagedorn, T. J. Stevenson, and S. S. Seefeldt. 2016. Field degradation of aminopyralid and clopyralid and microbial community response to application in Alaskan soils. Environmental Toxicology and Chemistry **35**:485-493.

Due to the cold temperatures at high latitudes there is concern that aminopyralid and clopyralid may persist longer in soil than in warmer regions where degradation occurs rapidly. Tomco et al. (2016) sprayed plowed agricultural fields with aminopyralid or clopyralid at the recommended rates. Both herbicides degraded rapidly over the first 29-period following the herbicide applications. After 90 days, residual clopyralid was <0.02μg/g at one field site and 0.046 μg/g at the other. Aminopyralid was slightly more persistent with 0.049 μg/g at one site and 0.12 μg/g at the other site. No differences in microbial diversity or community composition was found in treated and untreated soil the herbicides.

EFFICACY

Annen, C. A., J. A. Bland, A. J. Budyak, and C. D. Knief. 2018. Evaluation of Clopyralid and Additives for Coronilla varia Suppression in a Remnant Prairie (Wisconsin). Ecological Restoration **36**:111-113.

Annen et al. (2018) evaluated the effectiveness of clopyralid (Transline) to control crown vetch (*Coronilla varia*) in a midwestern grassland. They also evaluated the effects of the herbicide on non-target plant species richness. Clopyralid was blended with a methylated seed oil/nonionic surfactant and was applied with backpack sprayers at 0.4% (0.5 ounces/gallon, or 4 mL/liter). The herbicide was applied annually, in June, for 4 years. After 4 years the mature plants were successfully killed, but seedlings were emerging. Species richness of non-target plants was 27 in the treated section and 10 in the untreated section. Annan et al. (2018) concluded that clopyralid is an effective, cost-feasible means to control crown vetch and promote native prairie vegetation, provided a multiple-year effort is employed.

Coburn, C. W., A. T. Adjesiwor, and A. R. Kniss. 2018. Creeping Bellflower Response to Glyphosate and Synthetic Auxin Herbicides. Horttechnology **28**:6-9.

Coburn et al. (2018) compared the efficacy of 5 different herbicides (glyphosate, dicamba, clopyralid, quinclorac, and triclopyr) on greenhouse-grown creeping bellflower (*Campanula rapunculoides*). The herbicides were applied at five rates. Clopyralid caused greater creeping bellflower biomass reduction and mortality than the other herbicides investigated. The herbicide dose required to cause 50% mortality was lowest for clopyralid (86–138 g/ha) compared with dicamba (221–536 g/ha), glyphosate (196–678 g/ha), triclopyr (236–782 g/ha), and quinclorac (>3000 g/ha.).

DiTomaso, J. M., G. B. Kyser, D. J. Lewis, and J. A. Roncoroni. 2017. Conventional and Organic Options for the Control of Woolly Distaff Thistle (Carthamus lanatus). Invasive Plant Science and Management **10**:72-79.

DiTomaso and et al (2017) conducted two field experiments to evaluate the efficacy of different control options for *Carthamus lanatus* (Woolly distaff thistle). *Carthamus lanatus* is a long-lived winter annual that occurs on in coastal counties in Northern California. They compared several conventional herbicides at two timings and rates. They also compared a conventional herbicide treatment with organic and organic control methods, including an organic herbicide (mixture of capric and caprylic acids). Results of the conventional herbicide treatments showed most spring applications (March or April) of aminopyralid, aminocyclopyrachlor, clopyralid, and combinations of aminopyralid + triclopyr, or aminocyclopyrachlor + chlorsulfuron had greater than 99% control of *Carthamus lanatus,* with fewer than 1.5 seedlings per 27-m2 plot by the end of the growing season. Higher rates were generally necessary to achieve the same level of control with winter (January) applications. In the organic herbicide treatments, the most consistent treatment was a combination of mowing followed by 9% (v/v) or the organic herbicide. This treatment was slightly less effective compared with aminopyralid but did have better than 95% control of *Carthamus lanatus*. The results of this study provide control options for both conventional and organic ranching practices where woolly *Carthamus lanatus* is a problem.

Jorgensen, N. A., M. J. Moechnig, M. B. Halstvedt, and M. J. Renz. 2017. Native Forb Establishment following Application of Aminopyralid or Clopyralid. Invasive Plant Science and Management **10**:90-98.

Jorgensen et al. (2017) evaluated the effects aminopyralid and clopyralid on seeded non-target common forb species. They applied aminopyralid (54 or 123 g/ ha), clopyralid (237 and 420 g/ ha), or aminopyralid + clopyralid (54 + 237 g/ ha) in July and then seeded the treatment areas the following fall and spring. The herbicide treatments did not affect seedling establishment. Results suggest native forbs can tolerate these herbicides when applied at least 4 months prior to seeding.

Lym, R. G., R. L. Becker, M. J. Moechnig, M. B. Halstvedt, and V. F. Peterson. 2017. Native Grass Establishment following Application of Pyridine Herbicides. Invasive Plant Science and Management **10**:110-117.

Lym et al. (2017) evaluated the effects of aminopyralid, clopyralid, or picloram applied in the fall to areas seeded the following fall or spring with native cool and warm season grasses. The herbicides were applied at standard rates. Aminopyralid did not reduce seedling establishment in either fall or spring seeding. For the most part none of the herbicide applications affected seedling establishment of most of the seeded grasses. The exceptions were a slight reduction in fall-seeded wheatgrass in the clopyralid and picloram treatments and a Canadian wild rye in the picloram treatment.

oyd, and P. J. Dittmar. 2016. Clopyralid Dose Response for Two Black Medic (Medicago lupulina) Growth Stages. Weed Technology **30**:717-724.

Sharpe et al. (2016) compared the efficacy of clopyralid applied to small or large *Medicago lupulina* plants. Clopyralid adequately controlled *Medicago lupulina* when applied at maximum label rates when plants were small, but not when plants were large.

Tran, H., K. C. Harrington, A. W. Robertson, and M. S. Watt. 2015. Relative persistence of commonly used forestry herbicides for preventing the establishment of broom (Cytisus scoparius) seedlings in New Zealand plantations. New Zealand Journal of Forestry Science **45**.

Tran et al. (2015) evaluated the relative persistence and effect over time of several different herbicide treatments on broom seed viability following an early summer application. Here I only describe the effect of the herbicides used by MROSD. Clopyralid and triclopyr were applied at the recommended rate for managing Scotch broom. These soils were collected periodically and sown with broom seeds. They found that the longer it had been since the herbicide had been applied the less it suppressed broom germination and growth.

Tran, H., K. Harrington, A. Robertson, and M. Watt. 2016. Assessment of herbicides for selectively controlling broom (Cytisus scoparius) growing with radiata pine (Pinus radiata) in New Zealand. New Zealand Journal of Forestry Science **46**.

Tran et al. (2016) evaluated several herbicide mixtures for use in controlling Scotch broom (*Cytisus scoparius*) in pine plantations. They applied 18 different herbicide treatments. The only herbicide treatments relevant to MROSD in this study were a low (half the recommended rate), medium (recommended rate) and high (2x recommended rate) applications of a clopyralid and aminopyralid mixture. The clopyralid /aminopyralid combinations gave good control of broom at all rates assessed. The highest rate of the clopyralid/triclopyr mix gave slightly better control than the medium rate.

ENVIRONMENTAL FATE

Palma, G., R. Demanet, M. Jorquera, M. L. Mora, G. Briceno, and A. Violante. 2015. Effect of pH on sorption kinetic process of acidic herbicides in a volcanic soil. Journal of Soil Science and Plant Nutrition **15**:549-560.

Palma et al. (2015) evaluated the influence of soil pH on the ability of tricopyr and clopyralid to bind to soil that contains high organic matter. For both of the herbicides the rate of adsorbstion decreased with increasing soil pH. However, tricopyr adsorbed to the soil far better than clopyralid did. Maximum adsorbtion occurred when soil pH was 4.0 with 69.7% adsorbition for triclopyr but only 11.7% for clopyralid. The differences in adsorbtion potential is relevant because herbicides not adsobed by the soil have a greater chance of leaching into water sources and potentially interacting with non-target organisms.

Munira, S., A. Farenhorst, K. Sapkota, D. Nilsson, and C. Sheedy. 2018. Auxin Herbicides and Pesticide Mixtures in Groundwater of a Canadian Prairie Province. Journal of Environmental Quality **47**:1462-1467.

Munira et al (2018) collected ground water samples from an agricultural area in Canada that grows canola, potatoes, wheat and barley. The samples were analyzed with a gas chromatograph to identify and determine the concentrations of herbicides in the groundwater. The concentrations of the herbicides detected never exceeded their respective individual maximum acceptable concentrations. Clopyralid detection was relatively even across seasons, with 33% of the total detections occurring in spring, 24% in summer, and 43% in fall. Clopyralid is typically applied in the fall, but it is known to take 12-79 days to achieve 90% degradation. The consistent detection of clopyralid across seasons could be the result of groundwater contamination in previous years because clopyralid is relatively stable in water. It could also be a result of localized applications of clopyralid in any of the seasons, or from the carryover of clopyralid in the soil from previous years and subsequent movement of clopyralid to groundwater in the year of sampling.

1.5 TRICLOPYR

HUMAN EXPOSURE AND HEALTH

Gilden, R., M. Plisko, K. Hiteshew, E. Friedmann, and D. Milton. 2016. Pesticide monitoring on soccer fields via shoe wipes and urine samples. Environmental Research **147**:294-296.

In a pilot study, Gilden et al. (2016) evaluated pesticide exposure in children that use an atheltic field that was sprayed with a herbicide formulation that contains several active chemicals, inlcuding triclopyr. Kids provided urine and shoe wipe samples that were nalyxed for pesticide residues. All chemicals, including triclopyr, were below the detection levels.

ECOLOGICAL TOXICITY

Curtis, A. N., and M. G. Bidart. 2017. Effects of chemical management for invasive plants on the performance of Lithobates pipiens tadpoles. Environmental Toxicology and Chemistry **36**:2958-2964.

Curtus and Bidart (2017) evaluated the effects of a high and low concentraion of tryiclopy on northern leapord frog (*Lithobates pipiens*) tadpoles. Triclopyr had no effect on tadpole survival but both concentrations reduce body mass by 16-24%.

Guilherme, S., M. A. Santos, I. Gaivao, and M. Pacheco. 2015. Genotoxicity evaluation of the herbicide Garlon((R)) and its active ingredient (triclopyr) in fish (Anguilla anguilla L.) using the comet assay. Environmental Toxicology **30**:1073-1081.

Guilherme et al. (2015) evaluated the genotoxic effects of Garlon and its active ingrediant triclopyr on the European eel (*Anguilla anguilla*). The fish were expose for either 1 or 3 days to Garlon or triclpyr at environmentally releveant concentrations. DNA damage occurred with both exposure durations and with both chemicals. Slightly more DNA damage was observed in fish exposed to Garlon than triclpyr alone.

Isbister, K. M., E. G. Lamb, and K. J. Stewart. 2017. Herbicide Toxicity Testing with Non-Target Boreal Plants: The Sensitivity of *Achillea millefolium* and *Chamerion angustifolium* to Triclopyr and Imazapyr. Environmental Management **60**:136-156.

Isbister et al. (2017) evaluated the sensitivity of yarrow (*Achillea millefolium*) and fireweed (*Chamerion angustifolium*), to imazapyr and triclopyr. These plants are non-target plants that frequently co-occur with target weeds under powerlines. Triclopyr caused extensive damage to yarrow at <50% of the maximum field application rate and was lethal to fireweed at the lowest dose tested. Both non-target species were extremely sensitive to imazapyr and the lowest doses tested caused >75% mortality.

Marileo, L. G., M. A. Jorquera, M. Hernandez, G. Briceno, M. D. Mora, R. Demanet, and G. Palma. 2016. Changes in bacterial communities by post-emergent herbicides in an Andisol fertilized with urea as revealed by DGGE. Applied Soil Ecology **101**:141-151.

In a mesocosm experiment, Marileo et al. (2016) studied the interacting effects of urea and post emergent herbicide additions on bacterial communities. They evaluated 6 herbicides (MCPA, flumetsulam, fluroxypyr, triclopyr, clopyralid and picloram). Since urea or other fertilizers are not generally applied in natural land management I will only describe the effects of the relevant herbicides applied without urea. In the absence of urea, significant changes in total bacteria between the triclopyr-treated and control soils were observed, but only when the 2-fold-higher recommended dose of triclopyr was applied (1.94 mg/kg soil) at day 15. Changes in the ammonium oxidizing bacterial communities were also observed between the control soils and those that were treated with the 2-fold-higher recommended dose of triclopyr. Clopyralid did not change total bacteria or ammonium oxidizing bacterial communities. Residual triclopyr concentrations decreased by 81.4% 15 days following the application and clopyralid decreased by 73.7%.

Souza-Alonso, P., A. Guisande, and L. Gonzalez. 2015. Structural changes in soil communities after triclopyr application in soils invaded by Acacia dealbata Link. Journal of Environmental Science and Health Part B-Pesticides Food Contaminants and Agricultural Wastes **50**:184-189.

Souza-Alonso et al. (2015) evaluated the effect of triclopyr on microbial soil communities. Triclopyr was sprayed on *Acacia dealbata* trees and soil communities were measured prior to the treatment and periodically for 18 months. Triclopyr changed the structure of soil bacterial communities, but not the fungal communities. The diversity of bacterial and fungal communities were not affected by triclopyr.

Suvarchala, G., and G. H. Philip. 2016. Toxicity of 3,5,6-trichloro-2-pyridinol tested at multiple stages of zebrafish (Danio rerio) development. Environmental Science and Pollution Research **23**:15515-15523.

Suvarchala et al. (2016) evaluated the effects of a metabolite of triclopyr on zebrafish embryos. Zebrafish were exposed to five concentrations of the metabolite (200, 400, 600, 800, 1000 µg/L). Fish embryos had increased mortality with concentration > 400 µg/L , and a delay in hatching time and decrease in percentage of hatched embryos. Reduction in heartbeat rate, blood flow and body and eye pigmentation was noticed in a dose dependent manner. Pericardial and yolk sac edema were most severe malformations caused by the metabolite.

Yahnke et al (2017) evaluated mortality risk, behavior, and body and liver condition, and time till metamorphose of red legged frogs (*Rana aurora*) exposed to triclopyr mixed with the surfactant, Competitor. Frogs were exposed to triclopyr for 96 hours and then monitored for 60 days. Triclopyr did not affect frog mortality, behavior, or body condition. Triclopyr exposed frogs did exhibit lethargy during the exposure period and had a 1-day delay in completing metamorphose. The authors conclude “Observed effects were minimal, especially compared with the potential for ecological impacts from unmanaged invasive plants.”

ENVIRONMENTAL FATE

Li, W., J. Mao, X. Dai, X. Zhao, C. Qiao, X. Zhang, and E. Pu. 2018. Residue determination of triclopyr and aminopyralid in pastures and soil by gas chromatography-electron capture detector: Dissipation pattern under open field conditions. Ecotoxicology and Environmental Safety 155:17-25.

Li et al. (2018) used a new method to evaluate the of triclopyr and aminopyralid residues in forage grass, hay, and soil and found that over time triclopyr and aminopyralid dissipated. In forage grass, both compounds degraded rapidly over the first 14- or 21-d period and at a slow rate over the remainder of experimental days. In soil, they degraded at a relatively slow rate, and dissipated steadily to below or close to the LOQ by 60-d post application. The half-lives of triclopyr were 1.4–1.8 d and 6.2–9.0 d and aminopyralid were 1.7–2.1 d and 8.2–10.6 d in terms of forage grass and soil, respectively. The residues concentrations 7 days following the treatments, the residues of aminopyralid and triclopyr in forage grass and hay were lower than maximum limits allowed by the EPA (USA).

Palma, G., R. Demanet, M. Jorquera, M. L. Mora, G. Briceno, and A. Violante. 2015. Effect of pH on sorption kinetic process of acidic herbicides in a volcanic soil. Journal of Soil Science and Plant Nutrition 15:549-560.

Palma et al. (2015) evaluated the influence of soil pH on the ability of tricopyr and clopyralid to bind to soil that contains high organic matter. For both of the herbicides the rate of adsorbstion decreased with increasing soil pH. However, tricopyr adsorbed to the soil far better than clopyralid did. Maximum adsorbtion occurred when soil pH was 4.0 with 69.7% adsorbition for triclopyr but only 11.7% for clopyralid. The differences in adsorbtion potential is relevant because herbicides not adsobed by the soil have a greater chance of leaching into water sources and potentially interacting with non-target organisms.

Tayeb, M. A., B. S. Ismail, and J. Khairiatul-Mardiana. 2017. Runoff of the herbicides triclopyr and glufosinate ammonium from oil palm plantation soil. Environmental Monitoring and Assessment 189.

Tayeb et al. (2017) evaluated triclopyr pollution in runoff from a palm plantation in Malaysia. Triclopyr was sprayed onto 0.5 ha of the plantation and runoff samples were collected for 120 days following the treatment. Only 0.025% of the amount of the triclopyr applied was recovered in runoff. These findings suggest that triclopyr in runoff is minimal due to of its short persistence and strong soil adsorption.

EFFICACY

Coburn, C. W., A. T. Adjesiwor, and A. R. Kniss. 2018. Creeping Bellflower Response to Glyphosate and Synthetic Auxin Herbicides. Horttechnology 28:6-9.

Coburn et al. (2018) compared the efficacy of 5 different herbicides (glyphosate, dicamba, clopyralid, quinclorac, and triclopyr) on greenhouse-grown creeping bellflower (*Campanula rapunculoides*). The herbicides were applied at five rates. The herbicide dose required to cause 50% mortality was lowest for clopyralid (86–138 g/ha) compared with dicamba (221–536 g/ha), glyphosate (196–678 g/ha), triclopyr (236–782 g/ha), and quinclorac (>3000 g/ha.).

DiTomaso, J. M., G. B. Kyser, D. J. Lewis, and J. A. Roncoroni. 2017. Conventional and Organic Options for the Control of Woolly Distaff Thistle (Carthamus lanatus). Invasive Plant Science and Management 10:72-79.

DiTomaso and et al (2017) conducted two field experiments to evaluate the efficacy of different control options for *Carthamus lanatus* (Woolly distaff thistle). *Carthamus lanatus* is a long-lived winter annual that occurs on in coastal counties in Northern California. They compared several conventional herbicides at two timings and rates. They also compared a conventional herbicide treatment with organic and organic control methods, including an organic herbicide (mixture of capric and caprylic acids). Results of the conventional herbicide treatments showed most spring applications (March or April) of aminopyralid, aminocyclopyrachlor, clopyralid, and combinations of aminopyralid + triclopyr, or aminocyclopyrachlor + chlorsulfuron had greater than 99% control of *Carthamus lanatus,* with fewer than 1.5 seedlings per 27-m2 plot by the end of the growing season. Higher rates were generally necessary to achieve the same level of control with winter (January) applications. In the organic herbicide treatments, the most consistent treatment was a combination of mowing followed by 9% (v/v) or the organic herbicide. This treatment was slightly less effective compared with aminopyralid but did have better than 95% control of *Carthamus lanatus*. The results of this study provide control options for both conventional and organic ranching practices where woolly *Carthamus lanatus* is a problem.

Enloe, S. F., N. J. Loewenstein, D. Streett, and D. K. Lauer. 2015. Herbicide Treatment and Application Method Influence Root Sprouting in Chinese Tallowtree (Triadica sebifera). Invasive Plant Science and Management 8:160-168.

Enloe et al (2015) compared the effectiveness of 6 different herbicides in controlling Chinese tallowtree (*Triadica sebifera*). They used triclopyr ester and triclopyr amine, aminocyclopyrachlor, aminopyralid, fluroxypyr, and imazamox for foliar, cut stump, and basal bark treatments in natural areas and measured root sprouting response to herbicide treatments. Aminocyclopyrachlor, aminopyralid, fluroxypyr, and imazamox all controlled Chinese tallowtree similar to, or better than, triclopyr. Aminocyclopyrachlor resulted in greater mortality of Chinese tallowtree than triclopyr did in foliar and basal bark studies, whereas aminopyralid was more effective than triclopyr in cut stump studies. Fluroxypyr resulted in greater mortality than triclopyr in cutstump and basal bark studies but not in foliar studies. Aminopyralid reduced total sprouting better than all other treatments in the cut stump study.

Enloe, S. F., S. E. O'Sullivan, N. J. Loewenstein, E. Brantley, and D. K. Lauer. 2018b. The Influence of Treatment Timing and Shrub Size on Chinese Privet (Ligustrum sinense) Control with Cut Stump Herbicide Treatments in the Southeastern United States. Invasive Plant Science and Management 11:49-55.

Enloe et al. (2018) evaluated the effectiveness of glyphosate and triclopyr applied to cut stumps of Chinese privet (*Ligustrum sinense*) compared with cutting alone in the spring and fall and across a range of size classes. Both glyphosate and triclopyr were very effective in controlling privet in spring and fall cut stump applications. Glyphosate was slightly more effective than triclopyr. Less sprouting occurred following November treatments relative to April treatments. Larger diameter stumps tended to sprout more than smaller stumps. These findings suggest privet can be effectively controlled by applying glyphosate or triclopyr to cut stumps in the spring or fall.

Griffiths, J., H. Armstrong, R. Innes, and J. Terry. 2018. Can aerial herbicide application control Grey Willow (Salix cinerea L.) and stimulate native plant recovery in New Zealand wetlands? Ecological Management & Restoration 19:49-57.

Griffiths et al. (2018) compared the efficacy of controlling grey willows in New Zealand wetlands with glyphosate and triclopyr. Glyphosate reduced Grey willow cover which resulted in increased cover of native wetland plants. Triclopyr was much less effective at controlling grey willow than glyphosate and did not result in increased cover of natives.

Harrington, T. B., D. H. Peter, and R. A. Slesak. 2018. Logging debris and herbicide treatments improve growing conditions for planted Douglas-fir on a droughty forest site invaded by Scotch broom. Forest Ecology and Management 417:31-39.

Harrington et al (2018) evaluated the efficacy of controlling Scotch broom with slash debris left in place following logging and applications of aminopyralid alone and in combination with triclopyr ester. Scotch broom cover decreased from 20% to 0% as a result of the logging debris and herbicide treatments and as a result soil moisture increased which improved the survival and growth of planted Douglas-fir.

James, T. K., and C. A. Dowsett. 2015. Herbicide responses of mat-forming weeds of forest remnants in New Zealand. New Zealand Plant Protection 68:1-6.

James and Dowsett (2015) compared the effectiveness of aminopyralid, triclopyr and aminopyralid + triclopyr in defoliating 6 different mat forming invasive plants (*Tradescantia fluminensis, Plectranthus ciliatus, Asparagus scandens, Hedera helix, Lamium galeobdolon* and *Selaginella kraussiana) that* limit native plant regeneration. They compared the speed, duration and extent of defoliation by the herbicides when applied at quarter, half and full label-recommended application rates for general weed control using a backpack sprayer. Low rates (338-675 g/ha) of triclopyr resulted in >90% defoliation of *T. fluminensis, A. scandens* and *L.*

galeobdolon, but a higher rate of 1350 g/ha only gave 85% defoliation of *H. helix*. Aminopyralid was effective against *S. kraussiana* but was very slow acting.

Knezevic, S. Z., O. A. Osipitan, M. C. Oliveira, and J. E. Scott. 2018. Lythrum salicaria (Purple Loosestrife) Control with Herbicides: Multiyear Applications. Invasive Plant Science and Management 11:143-154.

Knezevic et al. (2018) evaluated the effectiveness of 14 herbicide treatments for purple loosestrife (*Lythrum salicaria*) control over a 10-year period. Here I will describe only the treatments relevant to MROSD. Glyphosate was applied at 2.2 and 3.4 kg/ha, triclopyr at 1.3 and 2.1 kg/ha, and imazapyr at 1.1 and 1.7 kg/ha. The herbicides were applied every year until 100% control was achieved. The high application rates of glyphosate and imazapyr provided excellent purple loosestrife control (≥90%) that lasted at least a year following application, and by year 3 both herbicides had eradicated the invader. It took 9 years to eradicate purple loosestrife with triclopyr. The older the purple loosestrife, the more years of follow-up application were needed. Generally, there were higher percentages of grass cover following triclopyr applications and higher cover of broadleaf species following glyphosate or imazapyr treatments.

Tran, H., K. C. Harrington, A. W. Robertson, and M. S. Watt. 2015. Relative persistence of commonly used forestry herbicides for preventing the establishment of broom (Cytisus scoparius) seedlings in New Zealand plantations. New Zealand Journal of Forestry Science 45.

Tran et al. (2015) evaluated the relative persistence and effect over time of several different herbicide treatments on broom seed viability following an early summer application. Here I only describe the effect of the herbicides used by MROSD. Clopyralid and triclopyr were applied at the recommended rate for managing Scotch broom. These soils were collected periodically and sown with broom seeds. They found that the longer it had been since the herbicide had been applied the less it suppressed broom germination and growth

Weir, S. M., S. Y. Yu, A. Knox, L. G. Talent, J. M. Monks, and C. J. Salice. 2016. Acute toxicity and risk to lizards of rodenticides and herbicides commonly used in New Zealand. New Zealand Journal of Ecology **40**:342-350.

Weir et al. (2016) evaluated the toxicity of several herbicides and pesticides at varying concentrations on western fence lizards (*Sceloporus occidentalis*). The chemicals evaluated included 5 rodenticides: brodifacoum, coumatetralyl, pindone, diphacinone and cholecalciferol, and five herbicides: glyphosate, clopyralid, triclopyr, metsulfuron-methyl and haloxyfop-methyl. Here I only describe the results of chemicals included in the MROSD invasive species management plan. Pesticide risk was determined by comparing the 96-hour LD50s to oral exposure models, and when exposure exceeds toxicity, this represents risk. The LD50s was > 1750 μg/g for glyphosate and clopyralid, 550 for triclopyr, and > 1750 for Cholecalciferol. It is important to note that while we recorded lizard toxicity from triclopyr, the toxicity values were still quite high (LD50 = 550 µg g–1) and are probably environmentally unrealistic under normal application scenarios.

1.6 CLETHODIM

ECOLOGICAL RISKS

Lincoln, A. E., R. K. Brooks, and S. T. Hamman. 2018. Off-Target Impacts of Graminoid-Specific Herbicide on Common Camas (Camassia quamash) Growth, Abundance, Reproduction, and Palatability to Herbivores. Northwest Science 92:166-180.

Lincoln et al. (2018) evaluated how clothdim applied at different times of year, and at different frequecies, to control invasive grasses in PNW praries effects *Camas quamash*, a locally important forb. The herbicide treatment reduced leaf length and increased flower and seed production, but did not influence seed viability or palatability to herbivores. The observed effects suggest that clethodim is not detrimental to Camas and that repeated applications can be safely used in areas with high concentrations of Camas

Schultz, C. B., J. L. Zemaitis, C. C. Thomas, M. D. Bowers, and E. E. Crone. 2016. Non-target effects of grass-specific herbicides differ among species, chemicals and host plants in Euphydryas butterflies. Journal of Insect Conservation 20:867-877.

Schultz et al. (2016) investigated the non-target effects of clethodim on *Euphydryas colon* caterpillars. Numerous species in the *Euphydryas* genus are in decline worldwide. The effect of clethodim on caterpillar survival was inconclusive. However, clethodim exposure doubled the concentration of the aucubin in the caterpillars . Aucubin is a defense compound in the caterpillar host plant that is sequestered by the caterpillars. The increase found in the caterpillars likely reflects an increase in the concentration in the host plants in response to the herbicide. Changes in the abundance of this defense compound in the caterpillar may decrease predation.

ENVIRONMENTAL FATE

Sandin-Espana, P., B. Sevilla-Moran, C. Lopez-Goti, M. M. Mateo-Miranda, and J. L. Alonso-Prados. 2016. Rapid photodegradation of clethodim and sethoxydim herbicides in soil and plant surface model systems. Arabian Journal of Chemistry 9:694-703.

In a controlled lab study, Sandin-Espana (2016) evaluated the rate of photolytic degradation of clethodim**.** Photolytic degradation of clethodim was rapid in both plant tissue and water.

Jose Villaverde, J., B. Sevilla-Moran, C. Lopez-Goti, L. Calvo, J. Luis Alonso-Prados, and P. Sandin-Espana. 2018. Photolysis of clethodim herbicide and a formulation in aquatic environments: Fate and ecotoxicity assessment of photoproducts by QSAR models. Science of the Total Environment 615:643-651.

Jose Villaverde et al. (2018) investigated the photo degradation of clethodim in water. Clethodim degraded rapidly in water with the half-life of clethodim ranging from 27.9 minutes to 4.6 hours. Chemical products of clethodim degradation were more toxic to the bacteria *Vibrio fischeri* than the clethodim itself, and was most toxic when the herbicide was completely degraded. Clethodim degradation products, unlike the active substance, have the potential to be leached from soil.

EFFICACY

Enloe, S. F., and M. D. Netherland. 2017. Evaluation of three grass-specific herbicides on torpedograss (Panicum repens) and seven nontarget, native aquatic plants. Journal of Aquatic Plant Management 55:65-70.

Enloe and Netherland (2017) compared the effects of clethodim, a grass specific herbicide, with non-selective herbicides; glyphosate and imazapyr on target invasive grasses and non-target native aquatic forbs. Imazapyr and glyphosate reduced nongrass biomass by 64-100%, whereas clothdium did not affect native forbs at all. Clethodim, glyphoste and imazapyr all reduced the target grass cover by 69-85%.

Jeffries, M. D., T. W. Gannon, and F. H. Yelverton. 2017. Herbicide Inputs and Mowing Affect Vaseygrass (Paspalum urvillei) Control. Weed Technology 31:120-129.

Jefferies et al. (2017) evaluated several treatments to control vasseygrass *(Paspalum urvillei)*. These treatments evaluated the timing of mowing relative to the appplication of clethodim (and other herbicides). Mowing vassey grass 1 or 2 weeks prior to applying clethodim was the most effective control treatment and resulted in the lowest cover of the invader 52 weeks after the treatment.

Kleemann, S. G. L., C. Preston, and G. S. Gill. 2016. Influence of Management on Long-Term Seedbank Dynamics of Rigid Ryegrass (Lolium rigidum) in Cropping Systems of Southern Australia. Weed Science 64:303-311.

Kleemann et al. (2018) used clethodim to control rigid rye grass in (*Lolium rigidum*) in a pea crop system and found that the herbicide sprayed post emergence reduced the rigid rye grass seed bank after 3 years.

2.0 ADJUVENTS/SURFACTANTS:

Alcohol ethoxylate, Alkylphenol ethoxylate, Lecithin, Canola oil: ethyl & methyl esters

ECOLOGICAL RISKS

Crago et al. (2015) evaluated the effects of surfactants on male flathead minnow and rainbow trout that occur in the San Francisco Bay Delta. They used surfactant not They specifically tested 4 types of surfactants 1) non nonlyphenol, 2) 4-tert octyphenol, 3) octylphenol ethoxylates and 4) nonylphenol ethoxylates on estrogenic activity in these organisms. To the best of my (chemistry) knowledge the surfactants used at MROSD fall into these broader types. After 7-days of exposure there was no effect of the surfactants on the estrogenic activity of rainbow trout. However, the high concentration of the surfactants alone increased estrogenic activity in the minnow, but when these surfactants were combined with the herbicide 2-4,D there was no effect. Findings from this study suggest that endocrine systems of different aquatic species can vary in their response to surfactants, and that surfactants that have negative effects in isolation, will not necessarily have negative effects when combined with other chemicals.

Eivazi, F., N. Mullings, and M. L. Banks. 2018. Effect of Select Surfactants on Activities of Soil Enzymes Involved in Nutrient Cycling. Communications in Soil Science and Plant Analysis 49:371-379.

Eivazi et al. (2018) evaluated the effect of surfactants, herbicides, and surfactants and herbicide combinations on soil enzymes involved in nutrient cycling in two soils that differed in texture (i.e. clay content). The surfactant was a blend of alkylphenol ethoxylate and alcohol ethoxylate, both are used at MROSD. The surfactant was applied at the recommended rate and two times the field application rate. The surfactant had an inhibitory effect on enzymatic activity compared to the control but also increased microbial biomass

Wang, Y. N., Y. Zhang, X. Li, M. Z. Sun, Z. Wei, Y. Wang, A. A. Gao, D. Y. Chen, X. Zhao, and X. Z. Feng. 2015. Exploring the Effects of Different Types of Surfactants on Zebrafish Embryos and Larvae. Scientific Reports 5.

My knowledge of chemistry is not sufficient enough to understand if the fatty alcohol polyoxyethylene ether is similar to the surfactants used by MROSD. I included this paper because it demonstrates that a fatty alcohol surfactant can have negative impacts on zebra fish embryos. Wang et al. (2015) evaluated the effects of anionic [sodium dodecyl sulphate (SDS)], cationic [dodecyl dimethyl benzyl ammonium chloride] and non-ionic [fatty alcohol polyoxyethylene ether (AEO)] surfactants on zebrafish larval behavior. Five behavioral parameters were recorded using a larval rest/wake assay, including rest total, number of rest bouts, rest bouts length, total activity and waking activity. The results revealed that AEO at 1 µg/mL were toxic to larval locomotor activity and that SDS had no significant effects. They also tested the toxicities of the three surfactants in developing zebrafish embryos. AEO exposure resulted in smaller head size, smaller eye size and shorter body length relative to SDS and 1227. They further demonstrated that the developmental retardation stemmed from inhibited cell migration and growth. These findings provide references for ecotoxicological assessments of different types of surfactants, and play a warning role in the application of surfactants.

Rolando, C. A., R. E. Gaskin, S. F. Gous, D. B. Horgan, and L. G. Raymond. 2017. The Effect of Formulation, Dose, and Adjuvants on Uptake of Phosphite Into Pine Foliage. Plant Disease 101:1652-1658.

Rolando et al. (2017) tested whether or not of the adjuvant alcohol ethoxylate increases the adsoption of phosphite in pine needles. Phosphite is being evaluated as a possible fungicide to treat a fungal pathogen that causes red needle cast on *Pinus radicata*. This paper is poorly written and it is difficult to tell if the adjuvants improved uptake of the fungicide. My interpretation of the results are that the adjuvant effects are in conclusive.

3.0 FUNGICIDE

No studies were published between 2015 and 2018 that describe human health risks, ecological effects, or the environmental fate of the fungicide Phosphite K.

4.0 INSECTICIDES

4.1 DIATOMACEOUS EARTH

TO my knowledge no studies were published between 2015 and 2018 that describe human health risks, ecological effects, or the environmental fate of diatomaceous earth.

4.2 D-TRANS ALLETHRIN

To my knowledge no studies were published between 2015 and 2018 that describe human health risks, ecological effects, or the environmental fate of D-trans allethrin.

4.3 FIPRONIL

HUMAN HEALTH

Aerts, R., L. Joly, P. Szternfeld, K. Tsilikas, K. De Cremer, P. Castelain, J. M. Aerts, J. Van Orshoven, B. Somers, M. Hendrickx, M. Andjelkovic, and A. Van Nieuwenhuyse. 2018. Silicone Wristband Passive Samplers Yield Highly Individualized Pesticide Residue Exposure Profiles. Environmental Science & Technology 52:298-307.

Aerts et al. (2018) evaluated human pesticide residues on people . Pesticide exposure was highly individualized. Fipronil was detected on wrist bands worn by 10 out of 30 participants in concentrations that ranged from 4.8-90 ng/g. The participants in this study also filled out a survey that described their diets and demographics. Four of the participants that had fipronil exposure had pets that used a pet care product that contained fipronil. Smoking and vegetable rich diets were correlated with consumption with higher cumulative concentrations of pesticide residues in wristbands.

Hamsan, H., Y. B. Ho, S. Z. Zaidon, Z. Hashim, N. Saari, and A. Karami. 2017. Occurrence of commonly used pesticides in personal air samples and their associated health risk among paddy farmers. Science of the Total Environment 603:381-389.

Hamsan et al. (2017) investigated the exposure and health effects of several different chemicals used to increase rice productivity. Farmers that use fipronil breathed into tubes and the air samples were analyzed for the presence and concentration of fipronil (and the other pesticides). Fipronil was detected at 462.5 ng/m^3, this is lower than the concentration thought to be related to lifetime cancer risk.

Lu, M. Y., J. Du, P. X. Zhou, H. Chen, C. S. Lu, and Q. Zhang. 2015. Endocrine disrupting potential of fipronil and its metabolite in reporter gene assays. Chemosphere 120:246-251.

Lu et al. (2015) evaluated the effects of fipronil and its metabolite, fipronil sulfone, on the endocrine system. They found that fipronil and fipronil sulfone did not influence estrogenic processes. However, fipronil sulfone had a negative effect on thyroid hormone activity.

Mitchell, R. D., A. D. Wallace, E. Hodgson, and R. M. Roe. 2017. Differential Expression Profile of lncRNAs from Primary Human Hepatocytes Following DEET and Fipronil Exposure. International Journal of Molecular Sciences 18.

Mitchell et al (2017) investigated the effect of fipronil exposure on molecular pathways in the liver, specifically they evaluated changes in non-coding RNA. Non-coding RNA does not result in protein synthesis, but does perform other essential functions. When liver cells were exposed to 10 mM of fipronil 76 genes were upregulated and 193 were down regulated, that is out of 56,384 genes 269 behaved differently following fipronil exposure. It is unknown what, if any, effects these changes have on human health, these changes in gene expression could lead to changes in cellular function.

Ruangjaroon, T., D. Chokchaichamnankit, C. Srisomsap, J. Svasti, and N. M. Paricharttanakul. 2017. Involvement of vimentin in neurite outgrowth damage induced by fipronil in SH-SY5Y cells. Biochemical and Biophysical Research Communications 486:652-658.

Ruangjaroon et al. (2017) evaluated the effects of fipronil on cellular processes that are thought to be important in the development of neurodegenerative diseases. Fipronil induced structural changes in SH-SY5Y cells and shortened neurite outgrowth projections. These changes could lead to neurodegenerative diseases (e.g. Alzheimer’s, Parkinson’s, etc.). They conclude that fipronil is a risk factor for neuronal degeneration, which in the long-term could result in neuronal diseases.

ECOLOGICAL RISKS

AMPHIBIANS

Glinski, D. A., W. M. Henderson, R. J. Van Meter, and S. T. Purucker. 2018. Using in vitro derived enzymatic reaction rates of metabolism to inform pesticide body burdens in amphibians. Toxicology Letters 288:9-16.

Glinski et al. (2018) determined the amount of time it took for fipronil, and its metabolite, to leave the liver of toads following exposure to soil that had been sprayed with the maximum recommended application rate of fipronil. Fipronil concentration were measured in toad tissue at 2, 4, 12, 24- and 48-hours following exposure. Following exposure, fipronil decreased to nearly 0 within 24 hours, but its metabolite, fipronil sulfone, increased. This study does not attempt to place these results in context of toad health, making it hard to interpret the significance of these rates.

Gripp, H. S., J. S. Freitas, E. A. Almeida, M. C. Bisinoti, and A. B. Moreira. 2017. Biochemical effects of fipronil and its metabolites on lipid peroxidation and enzymatic antioxidant defense in tadpoles (Eupemphix nattereri: Leiuperidae). Ecotoxicology and Environmental Safety 136:173-179.

Gripp et al. (2017) evaluated the effects of fipronil and its metabolites on tadpole oxidative stress. Tadpoles were exposed to soil that was treated with 35, 120 and 180 μg/kg of fipronil. Though the concentrations used in this study were high, they were not lethal to tadpoles after 7 days of exposure. Most of the antioxidant enzymes evaluated in this study were not altered by fipronil(or its metabolites) in a dose dependent way.

Van Meter, R. J., D. A. Glinski, W. M. Henderson, A. W. Garrison, M. Cyterski, and S. T. Purucker. 2015. Pesticide Uptake Across the Amphibian Dermis Through Soil and Overspray Exposures. Archives of Environmental Contamination and Toxicology 69:545-556.

Van Meter et al. (2015) evaluated the effects of fipronil (and several other pesticides) that contacted green tree and barking frogs through either spray or through contact with treated soil. They added 1.1 ug/cm^3 to soil in an aquarium or sprayed the frogs directly. They measured pesticide concentrations in frog tissues after 8 hours of exposure. Prior to treating the frogs they dehydrated them for 24 hours so the skin would maximumly absorb the pesticides. The spray treatment resulted in higher concentrations of fipronil than contact through soil for both frog species. After 24 hours the sprayed barking tree frogs had 1.94 ppm of fipronil in their tissues and the green tree frog had 2.10 ppm. Fipronil concentrations administered through contaminated soil resulted in 0.57 and 0.21 ppm in barking frogs and tree frogs, respectively. It is unclear what the consequence of these consequences means for frog health.

Van Meter, R. J., D. A. Glinski, W. M. Henderson, and S. T. Purucker. 2016. Soil organic matter content effects on dermal pesticide bioconcentration in american toads (Bufo americanus). Environmental Toxicology and Chemistry 35:2734-2741.

Van Meter et al. (2016) applied fipronil to soil with high and low organic matter content then put toads in contact with the treated soils for 8 hours. Fipronil concentrations in toad tissues were greater when the toads had been on the the low-organic matter soil than the high-organic matter soil. Agricultural soils typically have relatively lower organic matter content and serve as a functional habitat for amphibians.

BEES AND BUTTERFLIES

Antunez, K., M. Anido, B. Branchiccela, J. Harriet, J. Campa, C. Invernizzi, E. Santos, M. Higes, R. Martin-Hernandez, and P. Zunino. 2015. Seasonal Variation of Honeybee Pathogens and its Association with Pollen Diversity in Uruguay. Microbial Ecology 70:522-533.

Antunez et al. (2015) evaluated how the presence and impacts of honeybee pathogens were influenced by added stress factors such as restricted pollen diversity and pesticides. They collected pollen from bees and then determined how much residue of a suite of pesticides contaminated the pollen. They determined that fipronil was not stored in pollen in any colony or season. This study is in contrast to similar studies and suggests that the risk of fipronil contamination varies between locations.

Bovi, T. S., R. Zaluski, and R. O. Orsi. 2018. Toxicity and motor changes in Africanized honey bees (Apis mellifera L.) exposed to fipronil and imidacloprid. Anais Da Academia Brasileira De Ciencias 90:239-245.

Bovi et al. (2018) evaluated lethal and sublethal impacts of fipronil on Africanized honey bees. They measured 24-hour LD50 values for both oral ingestion and contact. The ingestion LD50 was 0.223 μg/bee and the contact LD50 was 0.008 μg/bee. Fipronil was more toxic when administered by contact than by ingestion. Bees were also given 1/500th of the lethal dose of fipronil either orally or via contact. Both modes of exposure of this sublethal dose caused bees to take a longer amount of time to walk through the 50-cm track. This study does not describe whether or not these concentrations are likely to be encountered by bees in nature.

Cebotari, V., I. Buzu, O. Gliga, O. Postolachi, and N. Granciuc. 2018. Content of pesticide residues in the flowers of the Acacia and linden trees from the moldavian codri area. Scientific Papers-Series D-Animal Science 61:235-242.

This is a really poorly written paper, partly because of the authors are not English speakers. The poor writing leaves me skeptical on the rigor/quality of peer review that this manuscript received. None the less, the study is simple and the results are somewhat relevant. Cebotari et al. (2018) measured the concentration of pesticide residues in *Robinia pseudoacacia* and *Tilia platyphillos* flowers from a forest that is presumably near to agricultural land. Average concentrations of fipronil residues in *Robinia* flowers ranged from 00.003 mg/kg and 0.001 mg/kg in *Tilia* flowers. The detected concentrations of fipronil residues in the tree flowers was from 1.7 to 5 times lower than the maximum limits allowed according to EPA (US) and European Union standards.

de Morais, C. R., B. A. N. Travencolo, S. M. Carvalho, M. E. Beletti, V. S. V. Santos, C. F. Campos, E. O. de Campos, B. B. Pereira, M. P. C. Naves, A. A. A. de Rezende, M. A. Spano, C. U. Vieira, and A. M. Bonetti. 2018. Ecotoxicological effects of the insecticide fipronil in Brazilian native stingless bees *Melipona scutellaris* (Apidae: Meliponini). Chemosphere 206:632-642.

de Morais et al. (2018) evaluated foraging activity of a stingless bee species that was exposed to sub lethal concentrations of fipronil for 6, 12 or 24 hours. They measured changes in behavior, locomotion, and brain structure morphology. They determined that a sublethal exposure of fipronil alters the climbing speed of foraging bees. Bees exposed to fipronil also had lethargy, motor difficulty, paralysis, and hyperexcitation. This paper had a very long and informative introduction that describes the state of knowledge regarding fipronil impacts on bees.

Edwards, E., R. Toft, N. Joice, and I. Westbrooke. 2017. The efficacy of Vespex (R) wasp bait to control *Vespula* species (Hymenoptera: Vespidae) in New Zealand. International Journal of Pest Management 63:266-272.

Vespex is a pesticide that contains fipronil to control wasps. To determine of fipronil is entering honeybee hives Edwards et al (2018) measured these chemicals in worker bees, bee larva, honey and pollen. Of the 320 samples comprising honey, worker bees, bee larvae and pollen that were examined, only one worker bee from the wasp treatment area contained fipronil residue. The authors conclude that the use of Vespex traps to control wasps poses little to no risk of entering bee hives and should support bee populations by reducing wasp predation.

Holder, P. J., A. Jones, C. R. Tyler, and J. E. Cresswell. 2018. Fipronil pesticide as a suspect in historical mass mortalities of honey bees. Proceedings of the National Academy of Sciences of the United States of America 115:13033-13038.

In the 1990s there was a large die off of honey bees and neonicotinoids were thought to be responsible. Holder et al. (2018) make a strong argument that the bee die off was more likely due to fipronil. Fipronil is used as a seed coat on sun flower crops (and other crop seeds) and translocates to all plant tissues including flowers. Sun flower plants are in flower for several weeks providing bees constant exposure to fipronil during that time. Holder et al (2018) used a multi-pronged approach to investigate the likelihood of fipronil as a driving agent in bee mortality. They quantified the toxicity of fipronil and neonicotinoids, and then incorporated the mortality rates into a demographic model. They also fed bees non-lethal doses of fipronil and found that almost all the ingested fipronil was present 6 days later, suggesting that it can bioaccumulate with continued exposure.

Jacob, C. R. O., H. M. Soares, R. C. F. Nocelli, and O. Malaspina. 2015. Impact of fipronil on the mushroom bodies of the stingless bee *Scaptotrigona postica*. Pest Management Science 71:114-122.

Jacob et al. (2015) evaluated the effect of fipronil on the morphology of protocerebral structures in the brains of a stingless bee. The fipronil concentrations applied to the bees were 0.27, 0.54, and 1.08 μg/bee. Oral and topical exposure to all the concentrations resulted in increased changes in cell morphology.

Kairo, G., Y. Poquet, H. Haji, S. Tchamitchian, M. Cousin, M. Bonnet, M. Pelissier, A. Kretzschmar, L. P. Belzunces, and J. L. Brunet. 2017. Assessment of the toxic effect of pesticides on honey bee drone fertility using laboratory and semifield approaches: a case study of fipronil. Environmental Toxicology and Chemistry 36:2345-2351.

Kairo et al. (2017) evaluated the effects of fipronil on honeybee drone fertility. Bees were fed a sugar solution spiked with a 0.1 μg/L of fipronil. Drone survival, maturity, and semen volume were not affected by fipronil. However, fipronil decreased the amount of sperm in semen and increased sperm mortality rate.

Kairo, G., D. G. Biron, F. Ben Abdelkader, M. Bonnet, S. Tchamitchian, M. Cousin, C. Dussaubat, B. Benoit, A. Kretzschmar, L. P. Belzunces, and J. L. Brunet. 2017a. *Nosema ceranae*, Fipronil and their combination compromise honey bee reproduction via changes in male physiology. Scientific Reports **7**.

Kairo et al. (2017a) evaluated the effects of fipronil and the fungal parasite, *Nosema ceranae*, on fertility and reproduction of honey bee drones. When fipronil or *Nosema* exposure were added individually there was no effect on bee survival, maturity rates, semen volume, or sperm count. However, when both *the fungal parasite and* fipronil were added together, the survival rate dropped from approximately 70% to 40%. Physiological markers in semen were impacted by fipronil alone and in combination with the parasite suggesting that semen quality is affected by the pesticide.

Lunardi, J. S., R. Zaluski, and R. O. Orsi. 2017. Evaluation of Motor Changes and Toxicity of Insecticides Fipronil and Imidacloprid in Africanized Honey Bees (Hymenoptera: Apidae). Sociobiology 64:50-56.

The objective of this study was to determine the 24 hour LD50 values and evaluate motor changes in Africanized honey bee foragers exposed to lethal and sublethal doses of fipronil. Foraging honey bees were exposed by ingestion and contact to five different doses of fipronil for 24 hours and then bee mortality was counted. The motor activity of bees exposed by ingestion or contact to LD50 and sublethal doses (1/500th of the LD50) of both pesticides was assessed 4 hours after exposure. The ingestion and contact LD50 values were 0.0528 and 0.0054 µg/bee, respectively. Bees exposed to sublethal doses of fipronil through ingestion experienced impaired motor abilities compared to the control. Bees exposed through contact did not exhibit motor changes.

Munoz-Capponi, E. A., G. Silva-Aguayo, J. C. Rodriguez-Maciel, and M. J. Rondanelli-Reyes. 2018. Sublethal exposure to fipronil affects the morphology and development of honey bees, Apis mellifera. Bulletin of Insectology 71:121-130.

Munoz-Capponi et al. (2018) evaluated the effects of 6 months of exposure to 0.025 μg/g (approximately 0.00125 µg/bee) of fipronil on honey bee morphology and development. The fipronil treated bees had abnormal development of wings and antennae and smaller body sizes relative to the untreated control group.

Nicodemo, D., D. De Jong, L. G. Reis, J. M. V. de Almeida, A. A. dos Santos, and L. A. M. Lisboa. 2018. Transgenic corn decreased total and key storage and lipid transport protein levels in honey bee hemolymph while seed treatment with imidacloprid reduced lipophorin levels. Journal of Apicultural Research 57:321-328.

Nicodemo et al. (2108) compared the lifespan and protein content of honey bees that were fed diets that contained non-hybrid, hybrid, or transgenic corn, the seeds of which had been treated with fipronil or not. They found no difference in total protein content in bees that were fed corn grown from seeds treated with fipronil. Likewise, life longevity of honey bees was not affected by consuming plants grown from seed treated with fipronil.

Paris, L., M. Roussel, B. Pereira, F. Delbac, and M. Diogon. 2017. Disruption of oxidative balance in the gut of thewestern honeybee Apis mellifera exposed to the intracellular parasite *Nosema ceranae* and to the insecticide fipronil. Microbial Biotechnology 10:1702-1717.

Paris et al. (2017) infected bees with the fungal pathogen, *Nosea ceranae,* and chronically exposed them to fipronil at low doses for 22 days and evaluated the impacts of the stress agents on oxidative balance. Protein oxidation was significantly increased in the *N. ceranae* and the fipronil combination suggesting that fipronil increases health risks of the fungal parasite.

Pashte, V. V., and C. S. Patil. 2018. Toxicity and Poisoning Symptoms of selected Insecticides to Honey Bees (*Apis mellifera mellifera* L.). Archives of Biological Sciences **70**:5-12.

Pashte et al. (2018) determined honey bee LC50 value of fipronil to be 0.0125%. They also made visual observations of bee morphology and behavior of bees exposed to lethal doses of fipronil, but did not compare these quantitatively to a control.

Roat, T. C., S. M. Carvalho, M. S. Palma, and O. Malaspina. 2017. Biochemical response of the Africanized honeybee exposed to fipronil. Environmental Toxicology and Chemistry 36:1652-1660.

Roat et al. (2017) evaluated the abundance of important enzymes in Africanized honeybee tissues exposed to fipronil. Of the 3 types of enzymes measured, 1 was changed by exposer to 0.01 ng/bee of fipronil, while 2 enzymes were not affected.

Taylor-Wells, J., J. Hawkins, C. Colombo, I. Bermudez, and A. K. Jones. 2017. Cloning and functional expression of intracellular loop variants of the honey bee (*Apis mellifera*) RDL GABA receptor. Neurotoxicology 60:207-213.

Taylor-Wells et al. (2017) identified 3 different genetic variants of GABA receptors in honey bees. They cloned these genes into another insect and exposed these insects to different concentrations of fipronil to evaluate if they varied in their response to the insecticide. The variants were not different in their responses to fipronil.

Yasuda, M., Y. Sakamoto, K. Goka, T. Nagamitsu, and H. Taki. 2017. Insecticide Susceptibility in Asian Honey Bees (Apis cerana (Hymenoptera: Apidae)) and Implications for Wild Honey Bees in Asia. Journal of Economic Entomology 110:447-452.

Yasuda et al. (2017) compared the effects of fipronil between a wild bee species in Japan and published LD50 values for honeybees. They determined that the acute 48-hour LD50 value of fipronil on the wild bee was 0.0025 μg/bee and the literature they cite reports a 0.00065 for honeybees. These results suggest that not all bees have the same LD50.

Zaluski, R., L. A. Justulin, and R. D. Orsi. 2017. Field-relevant doses of the systemic insecticide fipronil and fungicide pyraclostrobin impair mandibular and hypopharyngeal glands in nurse honeybees *(Apis mellifera*). Scientific Reports 7.

Zaluski et al. (2017) evaluated the effect of field-relevant doses of fipronil alone and combined with another pesticide, pyraclostrobin, on mandibular and hypopharyngeal glands in nurse honeybees. Bees exposed to fipronil with and without pyraclostrobin had nearly 50% smaller mandibular glands. The pesticides did not influence the number of acini in the hypopharyngeal glands. The amount of brood and worker eggs were also not influenced by the pesticides and no pesticide residues were found in control patties.

INSECTS

Jinguji, H., K. Ohtsu, T. Ueda, and K. Goka. 2018. Effects of short-term, sublethal fipronil and its metabolite on dragonfly feeding activity. Plos One 13.

Jinguji et al. (2018) evaluated the effects of short-term exposure to sublethal concentrations of fipronil on predatory activity of a widespread dragonfly. They compared the quantity of captured prey and the length of time it takes to capture prey in dragonflies exposed to fipronil to a control group . Dragonflies were exposed to several concentrations of fipronil and fipronil sulfone (0.01, 0.1,1.0, 10, 100 and 1000 μg/L). Dragonflies consumed less prey when exposed to 100 and 100 ug/L of fipronil, while 0.01, 0.1, 1, and 10 ug/L had no effect on prey consumption. Whereas all concentrations of the fipronil sulfone treatments reduced prey consumption. They also report sublethal effects of feeding inhibition appear to lead to severe mortality and that the metabolites of fipronil have higher toxicity than fipronil.

Sugita, N., H. Agemori, and K. Goka. 2018. Acute toxicity of neonicotinoids and some insecticides to first instar nymphs of a non-target damselfly, Ischnura senegalensis (Odonata: Coenagrionidae), in Japanese paddy fields. Applied Entomology and Zoology 53:519-524.

Sugita et al. 2015 evaluated the sensitivity of a Japanese damselfly to fipronil. They exposed damselflies to 0.5, 1, 2, 4 or 8 ug/L of fipronil and determined the 24- hour EC50 was between 1.423–2.246 μg/L. They contrast the EC50 of the damselfly with Daphnia to demonstrate that not all insects have the same sensitivity to fipronil and suggest that this is considered by pesticide regulators.

BIRDS

Khalil, S. R., A. Awad, and H. H. Mohammed. 2017. Behavioral response and gene expression changes in fipronil-administered male Japanese quail (Coturnix japonica). Environmental Pollution **223**:51-61.

Khalil et al (2017) evaluated the effects of fipronil on Japanese quail reproduction. They implemented a in a 15-day study and then tracked quail recovery for 60 days following exposure. Fipronil reduced food consumption and body weight. Several factors related to reproduction were evaluated. They found that overall fipronil exposure had negative effects on reproductive traits in the male Japanese quail that can result in increased infertility. However, most of the fipronil impacts on reproductive traits recovered over 20-40 days following exposure. The birds were fed corn oil dosed with 1/5 the lethal dose (LD50) of fipronil. This dose is quite high and not likely encountered.

Lopez-Antia, A., M. E. Ortiz-Santaliestra, P. R. Camarero, F. Mougeot, and R. Mateo. 2015. Assessing the Risk of Fipronil-Treated Seed Ingestion and Associated Adverse Effects in the Red-Legged Partridge. Environmental Science & Technology 49:13649-13657.

Lopez-Anita et al. (2015) fed fipronil treated and untreated seed to red legged partridges, a farmland associated bird, and compared bird fecundity and offspring quality. Birds did not reject the treated seed, but did reduce the amount consumed. Consumption of fipronil treated seeds resulted in reduced coloration, body condition, and reproductive performance in adult birds and lower quality offspring.

Bonmatin, J. M., C. Giorio, V. Girolami, D. Goulson, D. P. Kreutzweiser, C. Krupke, M. Liess, E. Long, M. Marzaro, E. A. D. Mitchell, D. A. Noome, N. Simon-Delso, and A. Tapparo. 2015. Environmental fate and exposure; neonicotinoids and fipronil. Environmental Science and Pollution Research 22:35-67.

Bonmatin et al. (2015) reviewed and synthesized the state of knowledge regarding the environmental fate and routes of exposure for fipronil (and neonicotinoids). They determined that fipronil has a low potential for volatilization and that most likely it will only be present in gaseous form for a short period during spray applications.

Chagnon, M., D. Kreutzweiser, E. A. Mitchell, E. A. Mitchell, C. A. Morrissey, D. A. Noome, D. A. Noome, J. P. Van der Sluijs, and J. P. Van der Sluijs. 2015. Risks of large-scale use of systemic insecticides to ecosystem functioning and services. Environmental Science and Pollution Research 22:119-134.

Chagnon et al. (2015) describes potential ways that impacts of fipronil, neonicitinoids, and pesticides in general may have cascading impacts that could ultimately reduce ecosystem functions and services. This paper makes logical arguments that are not well supported by data. For example, the authors describe the known impacts of fipronil on pollinators and then argue that this likely reduces pollination of crop species. This is entirely plausible, but until we know this to be true through rigorous investigation, it remains speculation. This paper provides several ideas about how pesticides could reduce ecosystem services and these ideas warrant further study.

Gibbons, D., C. Morrissey, and P. Mineau. 2015. A review of the direct and indirect effects of neonicotinoids and fipronil on vertebrate wildlife. Environmental Science and Pollution Research 22:103-118.

Gibbons et al. (2015) reviewed 150 studies that describe direct and indirect effects of fipronil (and neonicotinoids) on vertebrate wildlife.

4.4 INDOXOCARB

ECOLOGICAL RISKS

de Araujo, T. A., M. C. Picanco, D. D. Ferreira, J. N. D. Campos, L. D. Arcanjo, and G. A. Silva. 2017. Toxicity and residual effects of insecticides on Ascia monuste and predator Solenopsis saevissima. Pest Management Science 73:2259-2266.

De Araujo et al. (2017) studied the synergistic effects of indoxacarb on a crop pest (brassica caterpillar) and its ant predator (*Solenopsis saevissima)*. Indoxacarb killed 100% of the treated caterpillars and none of the ants.

Ghelichopour et al. (2018) evaluated the effects of idoxacarb on common carp (*Cyprinus carpio*). The fish were exposed to 0 (control), 0.75, 1.5 and 3 ppm of indoxacarb over 21 days. Indoxacarb exposure caused decreased protein synthesis, possibly as a result of liver damage. The blood tests also revealed that the insecticide also impaired thyroid function.

Neuman-Lee, L. A., E. D. Brodie, T. Hansen, E. D. Brodie, and S. S. French. 2016. Comparing the Natural and Anthropogenic Sodium Channel Blockers Tetrodotoxin and Indoxacarb in Garter Snakes. Journal of Experimental Zoology Part a-Ecological Genetics and Physiology 325:255-264.

Tetrodotoxin is a naturally occurring toxin that is used as an antipredator defense in the rough-skinned newt (*Taricha granulosa).*The insecticide indoxacarb and this newt defense compound have a similar mode of action. Some populations of the common garter snake (*Thamnophis sirtalis)* have evolved a resistance to tetrodotoxin. Garter snake tolerance to tetrodotoxin only occurs in snake populations that co-occur with the newt. Nueman-Lee et al. (2016) evaluated snake tolerance to the pesticide. When snakes were injected with these chemicals they had a stronger stress response to the pesticide than the newt defense compound.

Pashte, V. V., and C. S. Patil. 2018. Toxicity and Poisoning Symptoms of selected Insecticides to Honey Bees (Apis mellifera mellifera L.). Archives of Biological Sciences 70:5-12.

Pashte and Patil (2018) applied a variety of pesticides to a sunflower crop while the sunflowers were in bloom. They placed bagged bees over individual flowers so that they were exposed to the pesticides. They recorded the proprtion of bees that suvived. They determined that 71 % of the bees exposed to 5 mL/L of indoxocarb with in 24 hours of the application were killed. Whereas only 11% of bees died that were exposed to the treated plants 9 days after the application occurred. One short coming with this study is that they do report findings for the control group, which makes interpretation somewhat difficult.

Pozzebon, A., P. Tirello, R. Moret, M. Pederiva, and C. Duso. 2015. A Fundamental Step in IPM on Grapevine: Evaluating the Side Effects of Pesticides on Predatory Mites. Insects 6:847-857.

4.5 PRALLETHRIN

In a piolet study, Hung et al. (2018) measured the presence and concentrations of insecticide residues in dust samples collected inside and outside of 56 homes. Prallethrin was found in 38.5 % of the indoor dust samples and in 18% of the outdoor samples. The higher frequency of prallethrin inside suggest that it is or was used inside of homes to control pests.

4.5 PHENOTHRIN

No studies were published between 2015 and 2018 that describe human health risks, ecological effects, or the environmental fate of phenothrin.

4.6 S-HYDROPRENE

No studies were published between 2015 and 2018 that describe human health risks, ecological effects, or the environmental fate of S-Hydroprene.

4.7 SODIUM TETRABORATE DECAHYDRATE

No studies were published between 2015 and 2018 that describe human health risks, ecological effects, or the environmental fate of sodium tetraborate decahydrate.

5.0 *RODENTICIDES*

5.1 CHOLECALCIFEROL

No relevant studies to report

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Amid, C., M. Olstedt, J. S. Gunnarsson, H. Le Lan, H. Tran Thi Minh, P. J. Van den Brink, M. Hellstrom, and M. Tedengren. 2018. Additive effects of the herbicide glyphosate and elevated temperature on the branched coral Acropora formosa in Nha Trang, Vietnam. Environmental Science and Pollution Research **25**:13360-13372.

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