Midpeninsula Regional Open Space
Integrated Pest Management Program

Pesticide 2019 Literature Review and Annotated Bibliography

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1.1 Introduction

The information provided in this report is a synthesis of findings published in 2019 that describe the effects of pesticides used by MROSD on non-target organisms, and their persistence in the environment as well as their efficacy at controlling invasive species.

1.2 Literature Review and Evaluation Process

I performed a comprehensive literature review of all the scientific papers published in 2019 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 in 2019. 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

2.1.1 Human Exposure and Health Risks

Described in this review are epidemiological studies that evaluate human health risks associated with glyphosate use. These studies compare disease prevalence in groups with different levels of glyphosate exposure. Not included in this review are mechanistic in vitro studies on human cell lines or studies on rats and mice that evaluated physiological pathways whereby glyphosate could result in disease. Although these studies can offer important insights into how pesticides could cause disease they are beyond the scope of this report. These types of studies typically do not use glyphosate concentrations that are relevant to exposure levels experienced by herbicide applicators or the general public and cannot address the effects of long-term and repeated exposure. Richardson et al (2019) published a review paper that describes the state of knowledge regarding these potential pathways.

There is a great deal of controversy over the carcinogenic risks of glyphosate. The International Agency for Research on Cancer (IARC) is a World Health Organizations (WHO) program that uses a panel of experts to evaluate the evidence for carcinogenicity of chemicals. Experts that contribute to IARC chemical risk evaluations, published in IARC Monographs, volunteer a minimum of two weeks to review scientific literature prior to an 8-day panel meeting in Lyon, France. The WHO vets each expert panelist to ensure that they do not have conflicts of interest and the panelists are well aware of the possible implications of their classifications. In 2015, IARC classified glyphosate as “probably carcinogenic to humans”. IARC’s intent is to classify potential hazards and ask “can it cause cancer under any circumstance”. The IARC classification system is not intended to provide risk assessment, which is done by regulatory agencies such as the U.S. Environmental Protection Agency (EPA). Risk assessment further incorporates expected levels of exposure. In 2017, in opposition with IARC classification, the EPA, a regulatory agency, classified glyphosate as “not likely to be carcinogenic to humans”. However, since the IARC classification there has been a flurry of lawsuits claiming that people who occupationally apply glyphosate at high rates and frequency are at a higher risk than the population on average of getting non-Hodgkin’s lymphoma.

IARCs decision to classify glyphosate as a "probable human carcinogen" was in part due to the discovery that Monsanto (now owned by Bayer) was caught interfering with the scientific literature by ghost-writing papers and reviewing research papers to manipulate messaging prior to being submitted for peer review. These conflicts of interest have tainted the scientific literature and contributed to the difficulty of interpreting the evidence published in the scientific literature. Interpretation of conflicting results is very challenging with this body of literature partly because it is difficult to sort out the conflicting interests and biases of the researchers. At this point, there is no consensus in the scientific literature on the relationship between glyphosate exposure and cancer risk. The most important and informative study to date informing human health risks associated with glyphosate-based herbicides is the Agricultural Health Study. This long-term cohort study includes over 50,000 pesticide applicators in the US, where approximately 80% of the participants have been exposed to glyphosate. In this study 575 out of 50,000 have or have had non-Hodgkin's lymphoma. Two studies were published in 2019 that evaluated non-Hodgkin's lymphoma risk associated with glyphosate exposure (Zhang et al. 2019 and Leon et al. 2019). Both of these studies re-evaluated the Agricultural Health Study (AHS) cohort data that was presented in Andreotti et al (2018). In brief, Andreotti et al found that glyphosate applicators were not at a higher risk of getting non-Hodgkin's lymphoma. In 2019, Zhang et al. published a metanalysis that combined the large Agricultural Health Study with 4 smaller case control studies and re-evaluated the risk associated with high exposure to glyphosate and non-Hodgkin's lymphoma. They concluded that that risk of non-Hodgkin's lymphoma is 41% higher if you are in the highest glyphosate exposure group. One major difference between the Andreotti et al 2018 analysis and the Zhang et al 2019 analysis is that Zhang et al teased out just the people that had had the highest duration and intensity of exposure. Though the Zhang et al finding appears alarming, using just the highest exposure group is a somewhat misleading approach. Using only the highest exposure group is evidence of cherry-picking, hunting for an effect, rather than *observing* without bias. Generally, it is preferable to look for patterns between exposure level and disease prevalence, so that you can determine if increased exposure results in increased risk. Zhang et al found that applicators with 5, 10, or 15 years of exposure had lower rates of NHL compared to the general population. Agricultural workers who were using glyphosate for over 20 years also had access to chlordane, parathion, and several other now banned chemicals and chemical resistant gloves were less frequently used. Because there was no accounting for these covariates it is impossible to disentangle the effects of glyphosate from these other chemicals. It is difficult to say what explains the difference between 15 years of exposure (lower risk) and 20 years of exposure (higher risk). If glyphosate was capable of increasing non-Hodgkin's lymphoma risk, we should see a dose-dependent relationship among the 500 cases of non-Hodgkin's lymphoma in the cohort. No such relationship was found. Applicators in the bottom quartile for exposure had the same risk ratio as applicators in the top quartile. This lack of a relationship between exposure intensity suggests that glyphosate is not likely associated with non-Hodgkin's lymphoma. That said, some MROSD herbicide applicators might be in the situation where they will have 20 years of exposure to glyphosate in their career. Given the difficulty of determining if there are health risks associated with 20 years of cumulative exposure applicators should be taking steps from day one, to minimize exposure by using adequate PPE.

In a study that was funded in part by IARC, Leon et al (2019) used data from 3 large cohort studies in the US, France, and Norway to generate relative risk ratios (AKA odds ratios) in order to provide further insights into the relationship between non-Hodgkin's lymphoma and pesticide use by agricultural workers. The data from the US was from the Agricultural Health Study (AHS) that was analyzed by Andreotti et al (2018) and Zhang et al (2019). Leon et al corroborates some of the findings of Andreotti et al (2018), that is, overall, they found no association between non-Hodgkin's lymphoma and occupational exposure to glyphosate. Leon et al further evaluated risk associated with specific subtypes of non-Hodgkin's lymphoma and concluded that there was an elevated risk of diverse large B-cell lymphoma with a relative risk ratio of 1.36 (CI= 1-1.85). However, the relative risk overlaps 1 and is therefore difficult to interpret and requires more data before conclusions should be drawn. There are some important differences between the Leon et al study and the Andreotti et al study. Leon et al treated glyphosate exposure as a binary (ever exposed vs. never exposed) variable and essentially asked "does ever being exposed to glyphosate increase your risk of non-Hodgkin's lymphoma?", whereas Andreotti et al evaluated extent of exposure and related that to cancer risk (as described above). The authors decision to use glyphosate exposure as a binary variable rather than consider exposure levels make it less informative. This study also included the cases that could not recall the frequency of glyphosate exposure, whereas the Andreotti et al study did not include the cases where frequency of exposure could not be recalled. A strength of the Leon et al study is that it includes a few additional years of data from the Agricultural Health cohort study and includes data from other studies in other countries. Andreotti et al (2018) used a more nuanced, robust, and informative approach than either of the two cohort studies published in 2019 (Leon et al. 2019, Zhang et al. 2019) or that related lifetime exposure to disease risk.

Whether you err on the side of caution and assume that glyphosate is a "probable human carcinogen" or you find that the evidence is insufficient to demonstrate carcinogenicity when exposure levels are included in risk assessments, it is important to consider the major differences between the frequency and rates of application that agricultural workers experience and rates and frequency that MROSD and other wildland weed workers experience. The accumulative exposure to glyphosate is dramatically different between agricultural and wildland weed workers. All of the studies to date only consider cancer risk associated with agricultural glyphosate use. The pesticide application techniques are vastly different.
It is also relevant to consider that the safety measures and PPE used by pesticide applicators today are more protective than what was practiced decades ago.

Although, glyphosate is thought to be poorly absorbed through the skin (Wester et al. 1991), to date, there is little data in how much glyphosate enters the body following contact. A literature review by Gillezeau et al (2019) compared urine glyphosate concentrations of occupational glyphosate users to the concentrations found in the general population. On average urinary concentrations of glyphosate in people occupationally exposed varied from 0.26 to 73.5 µg/L and 0.16 to 7.6 µg/L. in the general public. The large variability among individuals and across studies could be due to a number of factors including methodological study approaches, time between exposure and urine test, difference in kidney health and metabolisms, amount of PPE used, and intensity of glyphosate exposure. Since these important and confounding variables could not be accounted for in this review it is difficult to say whether or not, or how much more glyphosate enters the bodies of occupational users versus the general public. In a review paper, Solomon et al. (2019) reported that 0.7-5.4% of glyphosate the contacts an applicators skin or clothing could be absorbed and that these concentrations do not exceed acceptable daily intake levels considered safe by regulatory agencies such as the US-EPA. In a letter to the editor, Perry et al. (2019) analyzed frozen urine samples that were initially collected from from United States farmers in 1997 and 1998 and found that 39% of the samples from farmers that used glyphosate had detectable levels of glyphosate (mean concentration 4.04 μg/kg; range:1.3–12 μg/kg) and that 0 samples had detectable glyphosate among the non-glyphosate applicator samples. The concentrations of glyphosate in urine that Perry et al reported were consistent with levels reported in the occupational biomonitoring studies reviewed by Gillezeau etal. 2019. Detecting glyphosate in these 20-year-old samples demonstrates that glyphosate exposures among farmers was prior to the widespread planting of genetically engineered glyphosate tolerant crops first approved in 1996.

Smpokou et al (2019) used a case control study approach to evaluate relationship between pesticide exposure in sugar cane farmers and Mesoamerican Nephropathy (kidney disease). They measured glyphosate (and other pesticides) in urine samples from 350 men and women between the ages of 18-30 years old, without self-reported kidney disease risk factors. Smpokou conducted kidney function tests over two years and correlated these measures with pesticide concentrations in urine and found no difference in glyphosate concentrations in urine between the people with declining kidney function and those with stable kidney function. In the majority of urine samples (68% and 70%) glyphosate concentrations were below the limit of detection (0.1 ng/mL). In a similar study, Trasande et al (2019) evaluated the relationship between low levels of glyphosate exposure and kidney (renal) disease. They measured concentrations of glyphosate in urine, as well as 3 biomarkers for kidney function in children. They identified glyphosate in urine in 11% of the children that had been exposed to glyphosate. They found no association between glyphosate and kidney function biomarkers. Together these studies suggest that glyphosate exposure poses minimal risk to kidney health.

von Ehrenstein et al (2019) used a case control study to evaluate risk associated with early developmental exposure to 11 pesticides, including glyphosate, and autism prevalence in California's Central Valley. They used data from California Department of Developmental Service (DDS) to identify the cases of Autism Disorder, the most sever autism spectrum diagnosis. They randomly selected controls from birth records in the region and used a 10:1 control to case ratio. The authors determined exposure based on data from California Pesticide Use Reports. They used GIS to estimate whether pregnant moms and infants were within 2000 or 2500 m from where glyphosate was applied in an agricultural context. Exposure was 'ever exposed' compared to 'never exposed'. Odds ratios and 95% confidence intervals were calculated and used to determine risk. The authors concluded that the risk of autism disorder was associated with prenatal exposure to glyphosate (odds ratio 1.16, 95% confidence interval 1.06 to 1.27). However, to understand if glyphosate, per se, is associated with autism the study would ideally identify and evaluate cases that were exposed to glyphosate and not the other 10 agricultural chemicals in this study. That can be difficult to impossible to do because rarely (if ever) do farmers use only 1 pesticide. When the authors attempted to consider the other chemicals in the analysis the relationship between glyphosate exposure during pregnancy and autism was lost, but was maintained for exposure during the 1st year of life. The authors also found that the association of autism prevalence was greater when the radius around homes was extended from 2 to 2.5Km, but do not examine what the associations were for a smaller radius (e.g. 1km). Exposure should presumably be greater, not smaller, when the distance between homes and where the glyphosate is applied. Another notable issue with this study is that many important factors are likely to differ between cases and controls. For example, the test group differs from the control group by not only having increased pesticide exposure, but by all the demographic differences that distinguish urban populations from agricultural ones. It is also suspect that the authors find similar autism risks associated with all 11 pesticides they evaluated (both herbicides and insecticides) even though these pesticides vary drastically in chemical structure and mode of actions. The only commonality is that all of the pesticides are used agriculturally. The methodological flaws in this study are in many ways unavoidable, but they also limit ability to draw the conclusion that autism is associated with glyphosate exposure.

Solomon (2019) is an update on a previous review published in 2016 and describes the range of glyphosate concentrations found in the environment. Solomon et al puts these concentrations in the context of exposure risks to the general public, domesticated animals, pets, and most importantly glyphosate applicators. In all cases, measured and estimated glyphosate exposures experienced by humans and animals were less than reference doses that are considered safe. Solomon concludes that realistic glyphosate concentrations found in the environment do not pose any substantial risk to people.

I recommend that MROSD pesticide applicators receive yearly up to date information regarding health risks associated with glyphosate. The National Pesticide Information Center (NPIC) provides excellent informational videos and fact sheets that are updated regularly with pertinent information as it becomes available. Kaci Buhl the Director of NPIC has an informative video on YouTube (https://youtu.be/xEQVpKm921w) and fact sheet (http://npic.orst.edu/factsheets/glyphogen.html).

2.1.2 Non-target Effects

Here I describe studies published in 2019 that evaluated the effects of glyphosate on non-target organisms including: animals, microbes, 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.

2.1.2.1 Amphibians

There were six papers published in 2019 that evaluated the effects of glyphosate on amphibian mortality, development, physiology, reproduction, and behavior. Each of these studies are described in the annotated bibliography (Appendix 1). None of the studies published in 2019 evaluated amphibian taxa present in the general vicinity of MROSD's jurisdiction. Four studies published in 2019 assessed the effect of environmentally relevant concentrations of glyphosate on amphibian mortality and none of these studies found a relationship between glyphosate and amphibian toxicity. Amphibian toxicity testing is not typically required for ecological risk assessments because it has been demonstrated that fish have comparable sensitivities. Daam et al (2019) compiled toxicity values for fish and amphibians from the US-EPA ECOTOX database and compared tropical and non-tropical species and found that amphibians were less sensitive than fish to glyphosate toxicity and that the standard fish test species rainbow trout (*Oncorhynchus mykiss*) was more sensitive than the most sensitive amphibian test species.

In aquatic environments the maximum concentration of glyphosate found in lakes, ponds, and wetlands was 0.301 mg/L and 0.427 mg/L in ditches and drains (Battaglin et al. 2014). In this review I consider these concentrations as worst-case glyphosate exposure scenarios. In 2019, no studies reported environmentally relevant concentrations of glyphosate to cause amphibian mortality. All four of the studies that did evaluate acute toxicity of glyphosate-based herbicides reported LC50 values that were more than a magnitude higher than environmentally relevant concentrations (Babalola et al. 2019, Carvalho et al. 2019, Daam et al. 2019, Lajmanovich et al. 2019).

While not lethal to amphibians, sublethal impacts of glyphosate-based herbicides on growth, and development, behavior, physiology, and DNA damage could have important consequences for amphibian populations. At very high concentrations, the glyphosate formulations Roundup and Kilo max caused tadpole growth reductions, however theses herbicides cannot technically be classified as growth inhibitors because the concentrations used in this study greatly exceeded relevant concentrations (Babalola et al. 2019). In amphibians, corticosterone regulates metamorphosis induction. Gulf coast toad (*Incilius nebulifer)* tadpoles exposed to glyphosate did not elicit a hormone (corticosterone) response, alter tail morphology, or change behavior in response to the predator cues (Gabor et al. 2019). In contrast, Wilkens et al (2019) found that bullfrog corticosterone production was reduced following exposure to a glyphosate-based herbicide that contained 234 µg of glyphosate/L. Exposure to a herbicide that contained 234 µg glyphosate/L for seven days had no effect on bullfrog tadpoles (*Rana catesbeiana*) nutritional condition, plasma protein, glucose, uric acid, or 3 out of the 4 biomarkers used to assess oxidative stress, but the herbicide did increase triglycerides and cholesterol (Wilkens et al 2019). DNA damage occurred in toad tadpoles (*Rhinella arenarum*) exposed to 5 % and 10% of the 96-hour LC50 concentration of a glyphosate-based herbicide (Carvalho et al. 2019). However, it is important to consider that even the low concentration of 5% of 78.18 mg/L used in this study exceeds glyphosate concentrations realistically found in the environment by several orders of magnitude. In a separate study, Lajmanovich et al. (2019) found that following 22 days of exposure to 1.25 mg/L of Roundup DNA damage was not induced. Collectively these studies provide undisputable evidence that environmentally relevant concentrations of glyphosate are not lethal amphibians. In some cases, the studies that evaluated sub lethal effects of glyphosate produced contradictory results. This suggests that more research is needed to better understand how field applications of glyphosate impact amphibian physiology and growth and whether or not these sublethal effects result in population level impacts (i.e. influence birth or death rates).

2.1.2.2*a *Terrestrial Invertebrates

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. Insect abundance and diversity are declining globally and it is important to understand what role, if any, glyphosate application is playing in this decline. In 2019, 7 studies were published that examined the effects of glyphosate on the health, behavior and food web dynamics of terrestrial invertebrates (Crone et al 2019, Galin et al 2019, Hanger et al 2019, Marqus et al 2019, Niedobova et al 2019, Pochron et al 2019, Talyn et al 2019.

Crone et al. (2019) found that Western monarch butterfly (*Danaus plexippus plexip*pus) abundance was negatively correlated with costal development, glyphosate use, neonicotinoid use, breeding season temperature and breeding season drought. Trends in statewide glyphosate use and coastal development are so highly correlated (r = 0.91) that they were effectively substitutable in the models. This collinearity makes teasing apart the effects of development and glyphosate use impossible. The authors concluded that more experimental research is needed to understand the relationship between glyphosate use and monarch abundance.

Fruit flies (*Drosophila melanogaster*) fed glyphosate at the manufacturers recommended rate (2.8 mg/ml) had reduced fertility and lifespan (Galin et al. 2019). In another study, Talyn et al. (2019) found that there was no difference in life span or reproductive behavior of fruit flies that were fed organic corn, non-GMO corn, GMO Roundup resistant corn, and GMO Roundup resistant corn that had been sprayed with Roundup. The also determined that the Roundup LC_{50} for male fruit flies was 7.1 and 11.4 g/L for females and that the concentrations of glyphosate residues that they detected in the glyphosate treated corn was seven orders of magnitude lower than the LC_{50} for male fruit flies. Hagner et al (2019) hypothesized that in northern latitudes the potential effects of glyphosate-based herbicides on non-target soil organisms could be larger than at lower latitudes because of the shorter growing season. With decreased time to degrade glyphosate, the herbicide could potentially have stronger impacts on soil organisms. They compared the effects of 3 management approaches to treating weeds and found that killing plants by hoeing had much larger effects on soil fauna than glyphosate did. The effects of glyphosate were minor and transient and no glyphosate remains were found in the soil at the end of the experiment. This is the kind of study that is really helpful to land managers trying to understand the impacts of herbicide use relative to other potential management actions. Survival of Colorado potato beetle (*Leptinotarsa decemlineata*) larval was not affected by a glyphosate-based herbicide (Roundup), but did inhibit the Ldace1 gene expression which encodes AChE activity and increased the activity of a biomarker associated with oxidative stress, suggesting that glyphosate increased oxidative stress (Margus etal 2019).

There was one study published in 2019 that described the non-target effects of glyphosate on spider behavior. Niedobova et al 2109 determined that Roundup by itself had no effect on predatory activity of wolf spiders (*Pardosa spp.*).

Studies have found conflicting results regarding the effects of glyphosate-based herbicides on earthworms. There was only one study published in 2019 that evaluated glyphosate impacts on earthworms. Pochron et al. (2019) evaluated how initial earthworm body size and soil temperature influence the effect of Roundup on earthworm growth and survival. They found that in hot soil, large worms exposed to Roundup grew larger than large worms not exposed to Roundup, but that growth of large worms in cold soil was not affected by Roundup. The growth of small worms was not affected by Roundup in either hot or cold soil. This study provides further evidence the relationship between earthworms and glyphosate are highly context dependent.

Collectively the studies published in 2019 suggest that the impacts of glyphosate on terrestrial invertebrates is nuanced and context dependent. For example, fruit flies that were fed glyphosate directly had shorter lifespans and reduced fertility, but that fruit flies that were exposed to plants sprayed with glyphosate were not affected by the herbicide. Earthworms exposed to glyphosate in hot soils had increased growth, whereas in cool soil the glyphosate had no impact on the worms. Glyphosate did not affect predatory behavior of wolf spiders by itself, but did reduce predatory behavior when it was mixed with a surfactant. Glyphosate use on invasive plants in wildlands may have some negative effects on invertebrates, however invasive plants may also impact terrestrial invertebrate abundance and diversity.

2.1.2.2*b *Bees

Pollinator decline has spurred research on the effects of glyphosate on pollinator health, particularly honeybee health. Since MROSD does not use aircraft or large equipment approaches to apply herbicide and instead uses primarily hand-held backpack sprayers and other methods 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. Bees can then transport contaminated pollen and nectar into hives and expose the brood. There were four papers published in 2019 that evaluated the non-target effects of glyphosate-based herbicides on bees. All four studies found that the herbicides did not have any adverse direct or indirect effects on bee survival following acute or chronic exposure (Blot et al. 2019, Decio et al. 2019, Ulziibayar and Jung 2019). The glyphosate concentrations used in these studies exceeded environmentally relevant concentrations. Uliziibayer and Jung (2109) applied a glyphosate-based herbicide to a native bee at twice the manufacturers recommended rate and it had no effect on bee survival.

Blot et al. (2019) found that fifteen days of chronic glyphosate (or AMPA) exposure did not influence honey bee survival, food consumption, or parasite infection. Glyphosate, but not AMPA, decreased the abundance of two of the dominant taxa in honey bee guts and increased the abundance of *Lactobacillus* spp. Findings from this study are consistent with Motta et al. (2018) and provides compelling evidence that the gut microbiome of honeybees is influenced by glyphosate, but that it is seemingly inconsequential to bee survival. It is well understood that neonicotinoids have detrimental impacts on honeybee health and viability. Decio et al (2019) evaluated whether the combined effects of a neonicotinoid (thiametoxam) and glyphosate were worse than the effects of the neonicotinoid alone. They injected worker bees with the neonicotinoid with and without added glyphosate and measured bee survival and several splicing patterns associated with important genes (x box, elav, and dscam) involved in responding to stress. Neither honeybee survival or gene-splicing patterns were affected by the addition of glyphosate. It should be noted that glyphosate concentrations injected into the honey bees was the highest water-soluble concentration possible (23mM) and it was administered at this rate twice over a 48-hour period. Tome et al. (2019) exposed honeybees to a glyphosate-based herbicide during the entire larval feeding period with an artificial diet containing glyphosate at worse-case environmental concentrations previously recorded in pollen or wax obtained from managed honey bee colonies. Glyphosate had no effect on larvae survival, pupae survival, development time, body mass or hypopharyngeal or antenna morphology. Adult honey bees did not up or down regulate any of the 8 measured detoxification genes. Bee larva downregulated one of the 8 genes.

None of the studies published in 2019 found any evidence that glyphosate has negative impacts in bee survival, development, stress, morphology, or nutrition.

2.1.2.3 Aquatic Invertebrates

Aquatic invertebrates could potentially be more vulnerable to pesticides than terrestrial invertebrates since pesticides can collect and concentrate in waterways. In 2019, only four studies were published that evaluated non-target effects of glyphosate on aquatic invertebrates (Canosa et al. 2019, de Melo et al. 2109, Marineau et al. 2019, Pala et al. 2019). Marinaeu et al (2019) found no difference in invertebrate abundance or diversity in the San Joaquin River Delta pre and one-month post glyphosate applications used to control water hyacinth. The authors conclude that current glyphosate treatments used to control water hyacinth poses minimal risk to aquatic invertebrates. de Melo et al (2019) found that gene expression and the production of a hormone involved in crustacean molting were unaffected by glyphosate in female shrimp, but that the male shrimp upregulated the molting genes after 7 hours of exposure to an intermediate glyphosate concentration, but not at higher or lower concentrations. The lack of a dose response pattern in males makes it difficult to infer implications, but suggests that negative result is spurious. One of the studies published in 2019 found that glyphosate can inhibit the nervous system and cause oxidative stress to *Gammarus pulex*, a freshwater amphipod (Pala et al. 2019), and another study found that the estuarine crab (*Neohelice granulata*) exposed to pure glyposate and a Roundup formulation for 30 days had reduced size and less muscle protein, and that Roundup, but not pure glyphosate, reduced spem count and inhibited the secreation of hormone from the the androgenic glad (Canosa et al 2019).

Together the studies publihed in 2019 provide further evidence that glyphosate can have sublethal non-target effects on aquatic invertabrates. Based on these findings, glyphosate applications near waterways should be used cautiosly and sparingly. As always, land managers must carefully weigh the potential costs to invertebrates against the known benefits of using glyphosate for invasive weed management. More studies that compare the impacts of herbicides to other management options are desperately needed to properly understand how wildland applications of glyphosate impact invertebrates and other non-target organisms. 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.

2.1.2.4 Reptiles

In 2019, only one paper was published concerning impacts of glyphosate on reptiles. Lus Hirano et al (2019) studied the effects of chronic exposure to 65 and 6500 ug/L of glyphosate on the bones and cartilage of turtle (*Podocnemis unifilis*) embryos and found that chronic glyphosate had no influence on the frequency or severity of bone or catildege malofmations or other skeletal abnormalities.

2.1.2.5 Birds

Two studies were published in 2019 that considered the non-target effects of glyphosate on birds. Fathi et al (2019) demonstrated that injecting eggs with glyphosate at a rate of 10 mg/Kg reduced the proportion of eggs that hatched by 66% and that the livers and kidney were enlarged in surviving chicks. This paper is not particularly relevant to wildland weed managers because it is not clear that bird chicks inside eggs would have any exposure to glyphosate in an invasive species control context. Rivers et al. (2019) used a large-scale field experiment to test how nest and post-fledging survival of white-crowned sparrows (*Zonotrichia leucophrys)* were influenced by herbicide intensity within managed conifer plantations across 2 breeding seasons. They found no evidence that either daily nest survival or post fledgling survival were influenced by light, moderate, or intensive use of glyphosate applied to forestry plantations. This paper has direct utility for land managers because it measured herbicide impacts following realistic concentrations in natural settings and because the researchers measured vital rates that ultimately contribute to population level persistence.

2.1.2.6 Soil Microbes

The effect of glyphosate on soil microbial communities can depend upon 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 apply glyphosate with large equipment or by aerial spray, and instead uses backpack sprayers and other methods that apply the herbicide directly onto the target plants it is very unlikely that glyphosate will be applied directly onto the soil. Since herbicides are not used when rain is forecasted at MROSD, it is unlikely to be rinsed from plants into the soil.

In 2019, four papers were published that evaluated the effects of glyphosate on soil microbial communities. Bruckner et al (2019) grew white clover (*Trifolium repens*) in pots with arbuscular mycorrhizal fungi. The clovers were sprayed with glyphosate and after two weeks microbial respiration increased by 30%, and there was a small shift in microbial community composition, with a decrease in the abundance of bacteria, and no effect on the abundance of mycorrhizal fungi. Unfortunately, this experiment did not include a treatment where the plants were killed by cutting without Roundup. It is not possible to distinguish between the effects of Roundup per se and plant/root death. We can however conclude from this study that glyphosate did not have any negative effects on mycorrhizal fungi. Farthing et al. (2019) compared the effects of different Bermuda grass (*Cynodon dactylon*) removal in grassland restoration projects. They determined that repeated glyphosate applications did not affect the species richness or diversity of the soil microbial communities. In another study, Gu et al. (2019) found that controlling weeds with Roundup reduced the total biomass of soil bacteria, fungi, and actinomycetes more than tilling or the untreated control treatments. Suleman et al. (2019) compared the abundance of 7 polar membrane lipids found in soil spiked with glyphosate. The lipids are proxies for microbial diversity. They found that of the 7 lipids identified in the soils, glyphosate applied at the maximum rate (1.25 kg/ha) decreased the concentration of 2 lipids, increased the concentration of 1 lipid, had no effect of 4 lipids. The lipid that was found in the highest abundance in the soil following the herbicide application was associated with fungi. It is unclear from these studies how shifts in the abundance and diversity soil microbes influences soil processes (e.g. decomposition rates, fertility, etc) or aboveground plant dynamics.

2.1.3 MANAGEMENT IMPLICATIONS

There is a rich body of literature dating back over 60 years that provides ample evidence that invasive species reduce biodiversity and alter ecosystem processes (Elton 1958, Richardson and Pysek 2008). Public agencies, such as MROSD are tasked with protecting biodiversity and preserving ecosystem integrity and the management of invasive species is a critical part of this responsibility. Controlling invasive species is extremely challenging and often times removal efforts are ineffective (Kettenring and Adams 2011). Controlling plants with herbicide is generally more effective than other approaches (Kettenring and Adams 2011). Given that there is far more information available on the non-target risks associated with glyphosate than any other herbicide, and that the risks appear to be quite minimal, using glyphosate over other herbicides is the safest option.

In agriculture, the intensity and frequency of glyphosate far exceeds its use in natural areas. In agriculture settings, pesticides, including glyphosate-based herbicides, can be applied to the same area, multiple times a year, and with mechanical equipment that cannot discriminate between target and non-target surfaces. These applications methods can result in a significant amount of spillover into the environment. Herbicide application techniques used by MROSD apply chemicals directly onto target plants and largely avoid spillover into the environment thus avoiding impacts to the non-target species that occur in these areas.

To date, the evidence clearly demonstrates that glyphosate is both highly effective at controlling invasive plants and that even the worst case environmentally relevant concentrations are not lethal to most animals that could inadvertently be exposed to the herbicide. Almost all of the studies published in 2019 that evaluated sublethal effects of glyphosate dosed animals with concentrations that far exceed what organisms are likely to encounter in even the worst-case scenarios that could occur in field situations. The numerous studies summarized in the MROSD reports suggest that sublethal impacts can sometimes occur. The non-lethal effect of glyphosate published in 2019 include changes to growth and development rates, behavior, and physiology. However, none of the studies further demonstrated that these sub-lethal effects resulted in population level consequences. These studies also do not evaluate how the sub-lethal effects recover over time following glyphosate exposure. Upon evaluation of the studies published in 2019 that evaluated non-target impacts of glyphosate, I see no compelling evidence that the use of glyphosate in natural areas poses substantial risk to the viability of non-target organisms. To date research suggests that the presence and spread of invasive species pose far greater risk to the conservation of biodiversity in natural areas than judicious use of glyphosate.

2.2 IMAZAPYR

No studies were published in 2019 that describe human health risks or the environmental fate of imazapyr. Two studies described non-target effects of imazapyr use on soil microbial processes and non-target plants (McCaskill et al. 2019 and Rohal et al. 2019). McCaskill et al. (2019) compared the effects of imazapyr (applied at at rate of .21 kg/ha) on soil nitrogen mineratlization, soil microbial biomass, and the survivl and growth of long leaf pine. Imazapyr was the only treatment that improved long leaf pine growth following a single application. One application of imazapyr increased nitrogen mineralization rates, and ammonification, but had no effect on microbial biomass. In another study, Rohal et al. (2019) expected native plant recovery to be less successful following imazapyr applications used to control phragmites than glyphosate applications because imazapyr has a longer half life than glyphosate and is purported to be able to persist longer in the soil. Contrary to their predictions, imazapyr did not restrict native plant recruit more than glyphosate. The authors speculate that this may be because the persistence of imazapyr in the anerobic soil conditions associated with wetlands is lower than in aerobicoic soil. There were five studies published in 2019 that describe the efficacy of imazapyr use to control invasive plants (Annen et al. 2019, Berrill and Howe 2019, Goodall and Braack 2019, Murray et al. 2019, Rohal et al. 2019) and are described in the annotated bibliography (Appendix1).

2.3 AMINOPYRALID

No studies were published in 2019 that describe human health risks, ecological effects, or the environmental fate of aminopyralid. One study was published in 2019 that describes the efficacy of aminopyralid use to control an invasive plant (Dias et al 2019) and is described in the annotated bibliography (Appendix1) .

2.4 CLOPYRALID

MacDonald et al. 2019 used a myriad of restoration approaches to increase cover of native forbs and grass in an area dominated by *Centaurea stoebe* (spotted knapweed). To remove C. stoebe prior to seeding with native forbs MacDonald et al sprayed the sites with 1) clopyralid or 2) glyphosate, or 3) mowed the sites. They found that clopyralid treated plots, and mowed plots had fewer native species than plots that were treatend with glyphosate.

2.5 TRICLOPYR

No studies were published in 2019 that describe human health risks or ecological effects of triclopyr. Only one study evaluated the environmental fate triclopyr in the environment. There were 4 studies published in 2019 that described triclopyr efficacy (Appendix 1).

To understand how agricultural applications of pesticides, including triclopyr, persit in estuaries Rodrigues et al. (2019) sampled surface water and sediments within an estuary in Portugal before and after the pesticides were applied to crops. Triclopyr was not found in concentrations that exceed the European Union’s acceptable limits in either the water or sediment samples. The authors conclude that the agruclтural pesticides used in the region, including tricolpyr pose minimal risk to aquatic orgamism that occur in the studied estuary.

2.6 CLETHODIUM

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

No studies were published in 2019 that describe human health risks, ecological effects, or the environmental fate of the adjuvants and surfactants used by MROSD.

4.0 FUNGICIDE

4.1 PHOSPHITE K

No studies were published in 2019 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 in 2019 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 in 2019 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 plants and animals is negligible. The research findings summarized in this report include studies that evaluate risk and best practices associated with bait station applications of fipronil. Fipronil bait stations are secure pesticide reservoirs that are placed where termites, roaches, or ants occur in or around structures. Currently PPE is not required during bait station application of fipronil. The potential handler and post-application exposure scenarios are minimal because of the low vapor pressure of fipronil, small treatment areas, and low application rates.

5.3.1 Human Exposure and Health Risks

In 2019, one study evaluated human exposure risk associated with fipronil. Harley et al. (2019) assesed weekly pesticide exposre in 14-16 year old latina girls that live in the Salinas Valley, an agricultual region of California. The study participants wore silicon wrist bands for a week that were analyzed for pesicide concentrations. Fipronil was detercted on 87% of the wrist bands and was the most frequntly deterced pesticide. In California, fipronil is not used in agricultue and is only used for treating termites, roaches, and ants in and around buildings, or to treat fleas and ticks on dogs and cats. The authors do not address what pathway the study participants were exposed to fipronil, but did find that living within 100 m of active agricultural fields, having carpeting in the home, and having an exterminator treat the home in the past six months were associated with higher odds of detecting certain pesticides.

No papers were published in 2019 that described the human health risks of fipronil.

5.3.2 Non-Target Effects

Pandit et al. (2019) used a microarray and qPCR approach to evaluate the effects of fipronil on the transcriptome profile associated with the lungs of mice that were fed 1/10th and 1/20th of the LC50 for 90 days. They concluded that these concentrations of fipronil can alter the transcriptome profile of lungs and potential cause damage through the production of cytokines that cause lung inflammation. Seydi et al. (2019) found that fipronil increased mitochondrial oxidative stress (increased production of ROS and LPO, mitochondrial swelling, and cytochrome C release) following apoptosis signaling. These impacts on mitochondria likely have hazardous effects on heart tissues. Testa et al. (2019) compared the amount fipronil in dust within homes with and without pets. They found that fipronil dust contamination in the presence of pets was far higher than in homes without pets. The amount of fipronil found in homes with and without pets never exceeded the concentrations considered hazardous by the US (EPA) or EU(ECHA) regulatory agencies. These papers are not directly relevant to MROSD because they do not assess the effects of fipronil bait stations used in and near to structures.

5.3.3 Environmental Fate

In 2019, 13 studies were published that evaluate effects of fipronil on non-target animals and X studies that describe 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

In 2019, one study evaluated human exposure risk associated with indoor use of indoxacarb bait traps. Wang et al (2019) measured residues of indoxocarb on kithchen and bedrooms floors of 69 low income apartments in New Jersey that used Advion indoxacarb bait traps and insect sticky traps to treat german cockroaches. In each apartment 18 g of gel bait (with 0.6% indoxacarb) was applied every two weeks for as long as cockroaches were still present. Tweleve months following the onset of the study indoxacarb was detectable in only 2 out of 69 apartments.

No papers were published in 2019 that described the human health risks of indoxacarb.

5.4.2 Non-Target Effects

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 were published in 2019 that evaluated the effects of indoxacarb on non-target organisms when indoxacarb is used in bait traps to control indoor pests. The European Food Safety Authority published a risk assessment that concluded that indoxacarb when used to control pests in corn crops posed high risk to small herbivorous after a single application and to earthworm eating mammals following repeated applications. They also concluded that adult honeybees and bumble bees are at high riskof being posisined if indoxacarb is applied to the crops prior to or during the flowering period, but not if applied after the flower period (Vernis et al. 2019).

5.5 PHENOTHRIN

No studies published in 2019 described the human health risks, ecological effects, or the environmental fate of phenothrin.

5.6 PRALLETHRIN

No studies published in 2019 described the human health risks, ecological effects, or the environmental fate of prallethrin.

5.7 S-HYDROPRENE

No studies published in 2019 described the human health risks, ecological effects, or the environmental fate of S-Hydroprene.

5.8 SODIUM TETRABORATE DECAHYDRATE

No studies were published in 2019 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 in 2019 that describe human health risks, ecological effects, or the environmental fate of cholecalciferol when it is used as a rodenticide.

Table 1: Summary of non-target effects of glyphosate published in 2019

Organism	***Findings published in 2019***	***Reference***
AMPHIBIANS		
Frog *(Xenopus laevis)*	The LC50 value of Roundup original for frog embryos exceeded concentrations found in some aquatic environments following treatment of aquatic plants. LC50 values of Kilo max and Enviro glyphosate did not exceed these environmental concentrations.	*Babalola et al. 2019*
Toad (*Anura bufonidae*)	The DNA of toad tadpoles that were exposed to 5% and 10% of the 96-hour LC50 concentration of glyphosate had altered DNA migration across an electrophoresis gel. DNA migration was a proxy for DNA damage. Even the low concentration of 5% of 78.18 mg/L used in this study exceeds glyphosate concentrations realistically found in the environment.	*Carvalho et al. 2019*
	Amphibians were less sensitive than fish to glyphosate toxicity and that the standard fish test species rainbow trout (*Oncorhynchus mykiss*) was more sensitive than the most sensitive amphibian test species.	*Daam et al. 2019*
Gulf coast toad *(*Incilius nebulifer)	Roundup did not elicit a hormone (corticosterone) response, alter tail morphology, or change behavior in response to the predator cues of Gulf Coast toad tadpoles.	*Gabor et al. 2019*
American toad (*Rhinella arenarum*)	Chronic exposure to Roundup did not affect Acetylcholinesterase (AChE), CbE, and GST activity in American toad tadpoles while acute exposure to 10 mg/L of Roundup increased AChE activity. Roundup had no effect on thyroid hormone levels in either the acute or chronic study. Roundup did not induce DNA damage or alter red blood cells.	*Lajmanovich et al. 2019*
Bullfrog (*Rana catesbeiana*)	A glyphosate-based herbicide had no effect on nutritional condition, plasma protein, glucose, or uric acid levels in bullfrog tadpoles. Triglycerides and cholesterol increased following herbicide exposure. In amphibians, corticosterone regulates the metamorphosis induction. Following exposure to glyphosate bullfrog corticosterone production was reduced. Four biomarkers were used to asses oxidative stress (SOD, CAT, TABRS and GST). Of these, only GST was affected by glyphosate.	*Wilkens et al . 2019*
FISH		
Cnesterodon decemmaculatus	Glyphosate had no effect on fish mortality. AChE was inhibited by exposure to 1 and 10 mg/l of glyphosate in all 4 seasons.	*Bernal-Rey et al. 2019*
Cnesterodon decemmaculatus	A glyphosate-based herbicide applied at rate of 0.2 and 2 mg/L for 42-days had no effect on genotoxicity or liver histology	*Bonifacio et al. 2019*
Zebrafish	Glyphosate had no effect on the time it took zebrafish to move into the top part of the tank, the frequency of crossing the tank,	*Chaulet et al. 2019*

Organism	***Findings published in 2019***	***Reference***
	or the total distance traveled, but it did cause the fish to rotate more frequently and to spend more time at the top of the tank. Implications from this study are that fish could be more vulnerable to predators if they are spending more time nearer to the surface.	
*Amur sleeper fish (*Perccottus glenii*)*	30-days of exposure to 2 ug/L Roundup reduced amolytic activity and water soluble protein content, but had no effect on body size, maltase, or AchE activity in Amur sleeper fish.	*Golovanova et al. 2019*
Rainbow trout (*Oncorhynchus mykiss*)	28 days of exposure to 1/5 or 1/10 of the LC50 concentration of Roundup decreased cerebral AChE activity and liver catalase activities.	*Meshkini et al. 2019*
	Physiological response of fish species to a glyphosate-based herbicide are different in lab and field conditoins and the physiological response of the fish varied between species. The findings collectively suggest that interpretation of lab studies may not be indicidive of risk in real world exposure sceanrios.	*Samanta et al. 2019*
REPTILES		
Turtle (*Podocnemis unifilis*)	Neither 65 and 6500 ug/L of glyphosate influenced the presence or severity of bone or catildege malofmations or any other skeletal abnormalities.	*Lus Hirano et al. 2019*
BIRDS		
White-crowned Sparrow (*Zonotrichia leucophrys*)	No evidence that either daily nest survival or post-fledging survival were influenced by herbicide application intensity.	*Rivers et al. 2019*
MAMMALS		
Cow	Even the highest concentrations glyphosate (10 mg/L) had no effect on the abundance of either *E. coli* or *Salmonella* in cow guts.	*Bote et al. 2019*
TERRESTRIAL INVERTABRATES		
Honey bee (*Apis mellifera*)	Neither glyphosate or AMPA had any effect on honey bee survival, food consumption, or *Nosema ceranae* parasite infection. Glyphosate, but not AMPA, decreased the abundance	*Blot et al. 2019*

Organism	***Findings published in 2019***	***Reference***
	of two of the dominant taxa in honey bee guts and increased the abundance of *Lactobacillus spp*.	
Honey bee (*Apis mellifera*)	Neither honey bee survival or gene-splicing patterns were affected by glyphosate injections. It should be noted that glyphosate concentrations injected into the honey bees was the highest water-soluble concentration possible (23mM) and it was administered at this rate twice over a 48-hour period.	*Decio et al. 2019*
Honey bee (*Apis mellifera*)	Glyphosate had no effect on larvae survival, pupae survival, development time, body mass or hypopharyngeal or antenna morphology. Adult honey bees did not up or down regulate any of the 8 measured detoxification genes. Bee larva downregulated one of the 8 genes	*Tome et al. 2019*
Bee (*Melipona scutellaris*)	Exposure to topically and orally applied glyphosate at half, twice, or the recommended dose, had no impact on bee survival.	*Uliziibayer and Jung 2019*
Fruit fly (Drosophila melanogaster)	The highest concentration (2.8 mg/ml) of glyphosate tested reduced the lifespan of male flies and the number of pupae and imago in the progeny, while the concentrations of 0.028 and 0.28 mg/ml produced no adverse effects. It should be notes that the highest concentration used in this study was the manufacturers recommended concentration for use in the environment.	*Galin et al. 2019*
	Removing invasive plants by hoeing had much larger effects on soil fauna than glyphosate did. The effects of glyphosate were minor and transient and no glyphosate remains were found in the soil at the end of the experiment.	*Hagner et al. 2019*
Pardosa spider (*Pardosa spp.*)	Roundup had no effect on spider behavior	*Niedobova et al. 2019*
Earthworm (Eisenia fetida)	In hot soil, large worms exposed to Roundup grew larger than large worm controls, but that growth of large worms in cold soil was not affected by Roundup. The growth of small worms was not affected by Roundup in either hot or cold soil. Worms in cold soil that were exposed to Roundup had lower survival than controls, but Roundup had no effect in hot soil.	*Pochron et al. 2019*
Fruit flies (*Drosphila melanogaster*)	There was no difference in life span or reproductive behavior of fruit flies that were fed organic corn, non-GMO corn, GMO Roundup resistant corn, and GMO Roundup resistant corn that was sprayed with Roundup. The concentrations of glyphosate residues that they detected in the glyphosate treated corn was seven orders of magnitude lower than the LC_{50} for male fruit flies.	*Talyn et al. 2019*

Organism	*Findings published in 2019*	*Reference*
AQUATIC INVERTEBRATES		
Estuarine crab (*Neohelice granulata*)	Exposed to Roundup and pure glyphosate for 30 days resultd in smaller crabs compared to a control group. These crabs had less muscle protein. Roundup, but not pure glyphosate, reduced spem count and inhibited the secreation of hormone from the the androgenic glad.	*Canosa et al. 2019*
Shrimp (*Macrobrachium potiuna*)	Male shrimp upregulated EcR genes after 7 hours of exposure to the 0.65 mg/L dose, but not the higher or lower herbicide concentrations. The lack of a dose response pattern makes it difficult to infer implications. Expression of the EcR gene in females was unaffected by glyphosate following 7 and 14 days of exposure. Vt transcription was upregulated in males following 14 days of glyphosate exposure of the intermediate concentration, but not with lower or higher herbicide concentrations. There was no effect of glyphosate-herbicide exposure on Vg expression of female shrimp. MIH also increased following 7-day glyphosate exposure for the intermediate concentration, but not the higher concentration, and not following 14-days of exposure.	*De Melo et al. 2019*
	There was no difference in invertebrate abundance or diversity pre and one-month post glyphosate applications.	*Marinaeu et al. 2019*
Amphipod (*Gammarus pulex*)	AChE activity in the freshwater amphipod was inhibited by 23% to 53% and oxidative stress increased when exposed to 10-40 ug/L of a glyphosate-based herbicide.	*Pala et al. 2019*
SOIL MICROBES	Glyphosate was applied to the plants and after two weeks microbial respiration increased by 30%. Glyphosate had a small effect on microbial community composition, decreased bacteria, and had no effect on mycorrhizal fungi. It is not possible to distinguish between the effects of Roundup per se and root death. Glyphosate and AMPA were detected in soil leachate, even though it had only been applied to the plants. This suggests that glyphosate entered the soil via the plants.	*Bruckner et al. 2019*
	Repeated glyphosate treatments had no effect on soil bacteria or archaea species richness or diversity.	*Farthing et al. 2019*
	Glyphosate applied at the maximum rate (1.25 kg/ha) decreased the concentration of 2 lipids, increased the concentration of 1 lipid, and had no effect of 4 of the lipids. The lipid that was found in the highest abundance in the soils is associated with fungi and algae and it decreased by a 10% as a result of the glyphosate addition.	*Suleman et al. 2019*

Organism	*Findings published in 2019*	*Reference*
AQUATIC MICROORGAMISMS	Following s glyphosate addition to brackish water microbial cell counts initially increased and the then decreased as the glyphosate was consumed by the microbes. After 71 days glyphosate was reduced by 99%. Glyphosate temporality increased the diversity and richness of the plankton and did not influence the diversity or richness of the biofilm.	*Janssen et al. 2019*
	At extremely high concentrations glyphosate inhibited growth of 2 plankton species and increased growth of the other. At environmentally relevant concentrations glyphosate had no effect on phytoplankton or cyanobacteria growth. Of the 3 herbicides tested, glyphosate was the least toxic.	*Lam et al. 2019*
PLANTS	Low concentrations glyphosate had no effect on the growth of a non-target plant (*Urochloa decumbens*), but that high glyphosate concentrations (>11 g/ha) decreased plant growth.	*de Moraes et al. 2019*
	Glyphosate reduced native plant biomass by 64-100%. Season of application did not influence the extent that the herbicides reduced native plant biomass.	*Enloe et al. 2019*
	Crop yield was unaffected by glyphosate or Roundup applications. In the greenhouse, oats germinated faster in the control soil than in the herbicide treated soils. Oat plants in the Roundup treated soil were smaller than plants in the untreated soil, but not the glyphosate soil. In the field, oats started out larger in the control, but by the end of the study were the same size in the control and herbicide treated soils. No traces of glyphosate or AMPA were detected in the greenhouse soils.	*Helander et al. 2019*
	Glyphosate were stored in root structures of perennial plants during dormancy periods and moved up to shoot and fruit portions in years following applications in some species	*Wood et al. 2019*

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Zhang, L., I. Rana, R. M. Shaffer, E. Taioli, and L. Sheppard. 2019. Exposure to glyphosate-based herbicides and risk for non-Hodgkin lymphoma: A meta-analysis and supporting evidence. Mutation Research-Reviews in Mutation Research **781**:186-206.

TABLE OF CONTENTS

1.0 HERBICIDES

1.1 GLYPHOSATE

HUMAN HEALTH

Crump, K. 2019. The Potential Effects of Recall Bias and Selection Bias on the Epidemiological Evidence for the Carcinogenicity of Glyphosate. Risk Analysis.

Crump (2019) evaluated the posisblility of bias intrinsic to the caluclation of odds ratios and realtive risk metrics that were reported in the 5 case control studies that have been conducted to assess cancer risk associated with glyphosate. Crump argues that the studies are at risk of recall bias and that two of these studies (Eriksson et al. 2008 and Hardell et al. 2002) are also at risk of selection bias. Crump further argues that these types of biases can make glyphosate appear carciocegenic even if it is not. In case control studies the evidence of glyphosate exposure relies on self reporting from the case and the control subjects. In theory this could reuslt in racall bias because the "cases" are more likely to "search their memories" more carefully to recall exposure scenarios whereas the "controls" may not be as motivated and fastidious at recalling exposure as those with the diesease. Selection bias could also be a problem because cases that do not claim exposure to glyphosate will be more likely than similarly unexposed controls to claim exposure to other pesticides. Thus, cases that claim no exposure to glyphosate will be more likely to be excluded from the analysis than controls who claim no glyphosate exposure.

Gao, H., J. Chen, F. Ding, X. Chou, X. Zhang, Y. Wan, J. Hu, and Q. Wu. 2019. Activation of the N-methyl-d-aspartate receptor is involved in glyphosate-induced renal proximal tubule cell apoptosis. Journal of Applied Toxicology 39:1096-1107.

Gao et al. (2019) used a cell culture model and an animal exposure model to evaluate 2 possible pathways that glyphosate could contribute to kidney disease. They investigated the influences of glyphosate on renal proximal tubule cells and the role that N-methyl-Daspartate receptor (NMDAR) has in the kidney's response to glyphosate exposure. They treated a human renal proximal tubule cell line with glyphosate for 24 hours at concentrations of 0, 20, 40 and 60 μM. In short, glyphosate reduced cell viability and induced apoptosis and oxidative stress in a dose-dependent manner. The mechanism appear to be in part due the expression of NMDAR1, as well as intracellular Ca2+, and reactive oxygen levels, increased after glyphosate treatment. Blocking NMDAR attenuated glyphosate-induced upregulation of Ca2+ and ROS levels as well as apoptosis. Inhibition of Ca2+ reduced glyphosate-induced reaction oxygen and apoptosis, and inhibition of ROS alleviated glyphosate induced apoptosis. In mice exposed to 400 mg/kg glyphosate, the urine low molecular weight protein levels started to increase from day 7. Upregulation of apoptosis and NMDAR1 expression in renal proximal tubule epithelium and an imbalance of oxidant and antioxidative products were observed. These results strongly suggest that activation of the NMDAR1 pathway, together with its downstream Ca2+ and oxidative stress, is involved in glyphosate-induced renal proximal tubule apoptosis.

Gillezeau C, van Gerwen M, Shaffer RM, Rana I, Zhang L, Sheppard L *et al.*, The evidence of human exposure to glyphosate: a review. *Environ Health* 18:2 (2019).

A literature review by Gillezeau et al. (2019) compared urine glyphosate concentrations of occupational glyphosate users to the concentrations found in the general population. On average urinary concentrations of glyphosate in people occupationally exposed varied from 0.26 to 73.5 µg/L and 0.16 to 7.6 µg/L. in the general public. The large variability among individuals and across studies could be due to a number of factors including methodological study approaches, time between exposure and urine test, difference in kidney health and metabolisms, amount of PPE used, and intensity of glyphosate exposure. Since these important and confounding variables could not be accounted for in this review it is difficult to say whether or not, or how much more glyphosate enters the bodies of occupational users versus the general public.

Hao, Y., Y. Zhang, H. Ni, J. Gao, Y. Yang, W. Xu, and L. Tao. 2019. Evaluation of the cytotoxic effects of glyphosate herbicides in human liver, lung, and nerve. Journal of Environmental Science and Health Part B-Pesticides Food Contaminants and Agricultural Wastes 54:737-744.

In this review paper Hao et al. (2019) examined the toxicity of glyphosate alone and glyphosate-based herbicides on liver, lung, and nerve tissues. The studies included in this review measured in vitro toxicity on human cell lines. They conclude that the toxic effects of glyphosate-based herbicides are primarily due to the use of formulants and that glyphosate salts alone are relatively safe.

Leon, M. E., L. H. Schinasi, P. Lebailly, L. E. B. Freeman, K.-C. Nordby, G. Ferro, A. Monnereau, M. Brouwer, S. Tual, I. Baldi, K. Kjaerheim, J. N. Hofmann, P. Kristensen, S. Koutros, K. Straif, H. Kromhout, and J. Schuz. 2019. Pesticide use and risk of non-Hodgkin lymphoid malignancies in agricultural cohorts from France, Norway and the USA: a pooled analysis from the AGRICOH consortium. International Journal of Epidemiology 48:1519-1535.

In this important paper, Leon et al. (2019) pooled cohort data from 3 large studies in the USA, France, and Norway to generate relative risk ratios in order to provide further insights into the relationship between non-Hodgkin's lymphoma and pesticide use by agricultural workers. This study was funded in part by the International Agency for Research on Cancer (IARC). Relative risk values greater than 1 with confidence intervals that do not overlap with 1 indicate that there is an association between non-Hodgkin's lymphoma and glyphosate. Overall, Leon et al did not find an association between having ever been exposed to glyphosate occupationally and risk of non-Hodgkin's lymphoma, that is the relative risk ratio was 0.95 (+/- 0.77.1.18). They further evaluated risk associated with specific subtypes of non-Hodgkin's lymphoma and concluded that there was an elevated risk of diverse large B-cell lymphoma with a relative risk ratio of 1.36 (+/1-1.85). However, the relative risk overlaps 1, barely, and is therefore difficult to interpret and requires more data before strong conclusions should be drawn. This is a big study and corroborates some of the findings of Andreotti et al. 2018, that it, overall there is no association between non-Hodgkin's lymphoma and glyphosate exposure. Some of the data in this study is the same data as in the Andreotti et al 2018 study, but with some major differences. Here Leon et al use the data to compare risk between ever exposed and never exposed and Andreotti et all evaluated extent of exposure and related that to risk. The Leon et al 2019 study also included the cases that could not recall the frequency of glyphosate exposure, whereas the Andreotti et al 2018 analysis did not include these cases. The Leon et al analysis also included a few additional years of data.

Martinez, A., and A. J. Al-Ahmad. 2019. Effects of glyphosate and aminomethylphosphonic acid on an isogeneic model of the human blood-brain barrier. Toxicology Letters 304:39-49.

Martinez et al. (2019) investigated the effect of acute exposure to concentrations ranging from 0.1 µM to 1000 µM of glyphosate on the blood-brain barrier. The short hand description of the methods described in this paper are beyond my capabilities to understand. In short, Martinez et all used an in vitro approach to manipulate pluripotent stem cells to evaluate how oral exposure to glyphosate, AMPA, and glycine influenced factors that could lead to blood brain barrier permeability. Although not statistically significant the authors found an increase in blood brain barrier permeability at very high (100 and 1000 µM) concentrations. They further concluded that glyphosate may increase blood brain permeability by the disrupting tight junctions. The authors did not address how realistic glyphosate exposure levels could influence blood brain barrier permeability.

Mendler, A., F. Geier, S.-B. Haange, A. Pierzchalski, J. L. Krause, I. Nijenhuis, J. Froment, N. Jehmlich, U. Berger, G. Ackermann, U. Rolle-Kampczyk, M. von Bergen, and G. Herberth. 2020. Mucosal-associated invariant T-Cell (MAIT) activation is altered by chlorpyrifos- and glyphosate-treated commensal gut bacteria. Journal of Immunotoxicology 17:10-20.

Mendler et al. (2019) investigated the effects of glyphosate on the ability for the 3 common bacteria species found in the human guts to produce vitamins (folate (b2) and riboflavin (B9)) and mucosal-associated invariant T-cells (MAIT). Gut microbiota are an important source of vitamins and humans cannot synthesize folate and riboflavin. MAIT cells produce pro-inflammatory cytokines and proteins. They found that when exposed to glyphosate, 2 out of the 3 bacteria species had increased production of MAIT, but that vitamin production was not influenced by glyphosate.

Perry, M. J., D. Mandrioli, F. Belpoggi, F. Manservisi, S. Panzacchi, and C. Irwin. 2019. Historical evidence of glyphosate exposure from a US agricultural cohort. Environmental Health 18.

In a letter to the editor, Perry et al (2019) reported data from a repository of frozen urine samples collected from United States farmers in 1997–98 in response to the recent review by Gillezeau et al (2019) published in Environmental Health. Of 18 glyphosate applicator samples tested, 39% showed detectable levels of glyphosate (mean concentration 4.04 µg/kg; range:1.3–12) compared to 0% detections among 17 non-glyphosate applicator samples (p-value < 0.01). Concentrations of glyphosate were consistent with levels reported in the prior occupational biomonitoring studies reviewed by Gillezeau et al. Accurately detecting glyphosate in this small sample of Wisconsin farmers demonstrates that glyphosate exposures among farmers were occurring 20 years ago, which was prior to the widespread planting of genetically engineered glyphosate tolerant crops first approved in 1996.

Richardson, J. R., V. Fitsanakis, R. H. S. Westerink, and A. G. Kanthasamy. 2019. Neurotoxicity of pesticides. Acta Neuropathologica 138:343-362.

Richardson et al. (2019) reviewed the literature and synthesized the studies that have evaluated the relationship between glyphosate (or the commercial formulations) exposure and neurotoxicity. The authors state that "While some research suggests that glyphosate inhibits AChE, the IC_{50} in human serum was calculated to be 714 mM, which is much higher than blood concentrations associated with indirect exposures (< 0.05 mM) or acute poisoning (0.05–5.0 mM). As such, this seems unlikely to be a mechanism of neurotoxicity. In terms of neuropathology, dopaminergic and γ-aminobutyric acidergic neurons preferentially undergo neurodegeneration in *C. elegans* treated with commercial glyphosate formulations at concentrations used by pesticide applicators. This neuro degeneration was attributed to mitochondrial inhibition and increased oxidative stress. Other studies demonstrate that zebrafish exposed to glyphosate formulations show abnormal brain development which may be attributable to glutamate excitotoxicity observed in developing rats exposed to glyphosate. More recently, rats treated with a commercial formulation showed increased anxiety and depression that correlated with changes in gut microbiota number and diversity. Since many bacteria also rely on the shikimic acid pathway to produce cyclic amino acids, inhibition of this pathway by glyphosate is hypothesized to decrease tryptophan catabolism. This decrease is potentially important since tryptophan is the precursor for serotonin, which plays an important role in both anxiety and depression. Taken together, these recent studies strongly support further research into the potential neurotoxic effects of glyphosate-based herbicides."

Smpokou, E.-T., M. Gonzalez-Quiroz, C. Martins, P. Alvito, J. Le Blond, J. Glaser, A. Aragon, C. Wesseling, D. Nitsch, N. Pearce, J. Norman, C. H. Lindh, J. Morton, and B. Caplin. 2019. Environmental exposures in young adults with declining kidney function in a population at risk of Mesoamerican nephropathy. Occupational and Environmental Medicine 76:920-926.

Smpokou et al. (2019) used a case control study approach to evaluate relationship between pesticide exposure in sugar cane farmers and Mesoamerican Nephropathy (kidney disease). They measured glyphosate (and other pesticides) in urine samples from 350 men and women between the ages of 18-30 years old, without self-reported kidney disease risk factors. Smpokou conducted kidney function tests over two years and correlated these measures with pesticide concentrations in urine. There was no difference in glyphosate concentrations in urine between the people with declining kidney function and those with stable kidney function. OTA and CIT were below the limit of detection. Glyphosate urinary concentrations were below the limit of detection (0.1 ng/mL) for the majority of the samples.

Solomon, K. R. 2019. Estimated exposure to glyphosate in humans via environmental, occupational, and dietary pathways: an updated review of the scientific literature. Pest Management Science.

Solomon (2019) is an update on a previous review published in 2016. This is a useful paper to read if you are trying to understand the range of glyphosate concentrations found in the environment and assess exposure risks to the general public, domesticated animals, pets, and most importantly glyphosate applicators. The levels reported in the literature are summarized here are also put into risk context. In all cases, measured and estimated systemic exposures to glyphosate in humans and animals were less than the acceptable daily intakes and the reference doses. Solomon concludes that realistic glyphosate concentrations found in the environment pose minimal risk to people

Szepanowski, F., C. Kleinschnitz, and M. Stettner. 2019. Glyphosate-based herbicide: a risk factor for demyelinating conditions of the peripheral nervous system? Neural Regeneration Research 14:2079-2080.

Szepanowski et al (2019) compared the effects of pure glyphosate and the glyphosate-based formulation Roundup LB plus on myelination and demyelination of neurites that would be associated with the peripheral nervous system. Glyphosate was added to culture medium before and after 10 days of myelination induction. Szepanowski et al found that glyphosate did not impact the neurons even at the highest concentrations. Glyphosate did not interfere with myelination or cause demyelination. However, there was a dose dependent effect of the Roundup LB Plus formulation on both myelination and demyelination and this appeared to be due to impairment of myelin rather than the integrity of the neurons. They conclude that the undisclosed additives in Roundup or likely driving these results.

Trasande, L., S. I. Aldana, H. Trachtman, K. Kannan, D. Morrison, D. A. Christakis, K. Whitlock, M. J. Messito, R. S. Gross, R. Karthikraj, and S. Sathyanarayana. 2020. Glyphosate exposures and kidney injury biomarkers in infants and young children. Environmental Pollution 256.

Trasande et al. (2019) evaluated the relationship between low levels of glyphosate exposure and kidney (renal) disease. They measured concentrations of glyphosate in urine, as well as 3 biomarkers for kidney function in children. They identified glyphosate in urine in 11% of the children that had been exposed to glyphosate. They found no association between glyphosate and kidney function biomarkers.

von Ehrenstein, O. S., C. Ling, X. Cui, M. Cockburn, A. S. Park, F. Yu, J. Wu, and B. Ritz. 2019. Prenatal and infant exposure to ambient pesticides and autism spectrum disorder in children: population based case-control study. Bmj-British Medical Journal 364.

Von Ehrenstein et al. (2019) used a case control study to evaluate risk associated with early developmental exposure to 11 pesticides, including glyphosate, and autism prevalence in California's Central Valley. They used data from California Department of Developmental Service (DDS) to identify the cases of Autism Disorder, the most sever autism spectrum diagnosis. They randomly selected controls from birth records in the region and used a 10:1 control to case ratio. The authors determined exposure based on data from California Pesticide Use Reports. They used GIS to estimate whether pregnant moms and infants were within 2000 or 2500 m from where the pesticides were applied in an agricultural context. Exposure was 'ever exposed' compared to 'never exposed' to the chemicals. Odds ratios and 95% confidence intervals were calculated and used to determine risk. If a odds ratio is greater than one and the confidence interval does not overlap 1 than risk is supported. If the odds ratio is 1 or below then no risk is assumed. The authors concluded that the risk of autism disorder was associated with prenatal exposure to glyphosate (odds ratio 1.16, 95% confidence interval 1.06 to 1.27). However, to understand if glyphosate, per se, is associated with autism the study would ideally identify and evaluate cases that were exposed to glyphosate and not the other 10 chemicals in this study. That can be difficult to impossible to do because rarely are farmers only using 1 pesticide. When the authors attempted to consider the other chemicals statistically the relationship between glyphosate exposure during pregnancy and autism was lost, but was maintained for exposure during the 1st year of life. The authors also found that the association of autism prevalence was greater when the radius around homes was extended from 2 to 2.5Km, but do not examine what the associations were for a smaller radius (e.g. 1km). Exposure should presumably be greater when the distance between homes and where the glyphosate is smaller. Another notable issue with this study is that many important factors are likely to differ between cases and controls. For example, the test group differs from the control group by not only having increased pesticide exposure, but by all the demographic and lifestyle differences that distinguish urban populations from agricultural ones. It is also suspect that the authors find similar risks associated with all the pesticides they evaluated (both herbicides and insecticides) even though these pesticides vary in chemical structure and mode of actions. The methodological flaws in this study are in many ways unavoidable, but they also limit ability to conclusively associate autism with glyphosate exposure.

Zhang, L., I. Rana, R. M. Shaffer, E. Taioli, and L. Sheppard. 2019. Exposure to glyphosate-based herbicides and risk for non-Hodgkin lymphoma: A meta-analysis and supporting evidence. Mutation Research-Reviews in Mutation Research 781:186-206.

Zhang et al. (2019) conducted a metanalysis approach that combined 5 case control studies and the large cohort study to re-evaluate the risk associated with high exposure to glyphosate and non-Hodgkins lymphoma. The analysis teased out just the subjects that had had the highest duration of exposure and the highest intensity of exposure. They found that that risk of non-Hodgkins lymphoma is 41% higher if you are in the highest glyphosate exposure group. Though the Zhang et al finding appears alarming, using just the highest exposure group is a somewhat misleading approach and is evidence of cherry-picking, hunting for an effect, rather than *observing* without bias. Generally, a more robust approach is to look for patterns between exposure level and disease prevalence, so that you can determine if increased exposure results in increased risk.

NON-TARGET IMPACTS

AMPHIBIANS

Babalola, O. O., J. C. Truter, and J. H. van Wyk. 2019. Mortality, teratogenicity and growth inhibition of three glyphosate formulations using Frog Embryo Teratogenesis Assay-Xenopus. Journal of Applied Toxicology 39:1257-1266.

Babalola et al. (2019) studied the effects of 3 formulations of glyphosate (Roundup original, Kilo max, and Enviro Glyphosate) on frog (*Xenopus laevis*) embryo survival, growth, and malformations. The LC50 of Roundup was 1.05 mg/L, Kilo max was 207 mg/L and Enviro glyphosate was 466 mg/L. Glyphosate concentrations found in aquatic environments following applications on aquatic plants can be as high as 1.43 mg/L. This suggests that Roundup original should not be used in aquatic environments where frog embryos are present. The other two glyphosate formulations are not toxic to the frog embryos. These findings suggests that something other than glyphosate in Roundup may have contributed to the embryo mortality rate. Although Roundup and Kilo max both caused tadpole growth reductions at very high concentrations, they cannot be classified as growth inhibitor. In order to be considered a growth inhibitor, embryo growth would have had to be suppressed at lower concentrations.

Carvalho, W. F., C. R. de Arcaute, J. M. Perez-Iglesias, M. R. R. Laborde, S. Soloneski, and M. L. Larramendy. 2019. DNA damage exerted by mixtures of commercial formulations of glyphosate and imazethapyr herbicides in Rhinella arenarum (Anura, Bufonidae) tadpoles. Ecotoxicology 28:367-377.

Carvalho et al.(2019) evaluated the toxicity of Credit, a glyphosate-based herbicide, and Pivot, an imazethapyr-based herbicide on bullfrog (*Anura bufonidae*) tadpoles. The 96-hour LC50 values were 78.18 mg/L and 0.99 mg/L for the glyphosate-based herbicide, and the imazethapyr based herbicide, respectively. Glyphosate was far less toxic than imazethapyr. Toad tadpoles were exposed to 5% and 10% of the 96-hour LC50 concentration of each chemical alone and in combination and changes in DNA migration across a gel (electrophoresis) was measured as a proxy for DNA damage. Both chemicals at both the 5% and 10% of the 96-hour LC50 values altered DNA migration across an electrophoresis gel. It is important to note that even the low concentration of 5% of 78.18 mg/L used in this study exceeds glyphosate concentrations realistically found in the environment.

Carvalho, W. F., C. R. de Arcaute, J. M. Perez-Iglesias, M. R. R. Laborde, S. Soloneski, and M. L. Larramendy. 2019a. DNA damage exerted by mixtures of commercial formulations of glyphosate and imazethapyr herbicides in *Rhinella arenarum* (Anura, Bufonidae) tadpoles. Ecotoxicology 28:367-377.

Carvalho et al. (2019) evaluated the genotoxicity of Credit, a glyphosate based herbicide used in Argentina. DNA damage (erythrocyte damage) was observed after the fish were exposed to 5% and 10% of Credit's 96-hour LC50. However, this paper is missing some important information. The authors do not describe how the fish were exposed to the herbicide or what the 96-hour LC50 is so it is difficult to evaluate the results because it is unclear if concentrations are relevant to concentrations observed in the environment.

Daam, M. A., M. F. Moutinho, E. L. G. Espindola, and L. Schiesari. 2019. Lethal toxicity of the herbicides acetochlor, ametryn, glyphosate and metribuzin to tropical frog larvae. Ecotoxicology 28:707-715.

Daam et al. (2019) evaluated the acute toxicity of glyphosate to tadpoles of two tropical frog species (*Physalaemus cuvieri* and *Hypsiboas pardalis*). The calculated 96-hour LC50 values for *P. cuvieri* and *H. pardalis* were 115 and 106 mg/L. They also compiled toxicity values for fish and amphibians from the US-EPA ECOTOX database and compared tropical and non-tropical species and found that amphibians were less sensitive than fish to glyphosate toxicity and that the standard fish test species rainbow trout (Oncorhynchus mykiss) was more sensitive than the most sensitive amphibian test species.

Gabor, C. R., H. R. Perkins, A. T. Heitmann, Z. R. Forsburg, and A. S. Aspbury. 2019. Roundup (TM) With Corticosterone Functions as an Infodisruptor to Antipredator Response in Tadpoles. Frontiers in Ecology and Evolution 7.

Gabor et al. (2019) exposed tadpoles to glyphosate and exogenous corticosterone to evaluate how these chemical interactions influence the behavior, morphology and stress hormone response of Gulf coast toad (*Incilius nebulifer)* tadpoles. Roundup alone did not elicit a hormone (corticosterone) response, alter tail morphology, or change behavior in response to the predator cues. The combined effects of exogenous corticosterone and glyphosate did increase activity following exposure to predator cues.

Lajmanovich, R. C., P. M. Peltzer, A. M. Attademo, C. S. Martinuzzi, M. F. Simoniello, C. L. Colussi, A. P. Cuzziol Boccioni, and M. Sigrist. 2019. First evaluation of novel potential synergistic effects of glyphosate and arsenic mixture on Rhinella arenarum (Anura: Bufonidae) tadpoles. Heliyon 5

Lajmanovich et al. (2019) evaluated the acute (48-hour) and chronic (22-day) exposure toxicity of a glyphosate-based herbicide alone and in combination with arsenic on common South American toad (*Rhinella arenarum*) tadpoles. Acetylcholinesterase (AChE), Carboxylesterase enzyme (CbE), and Glutathione S-transferase (GST) enzyme activity, thyroid hormones (triiodothyronine; T3 and thyroxine; T4) and DNA damage were examined following 48 hours of exposure 10 mg/L of the glyphosate-based herbicide or 22 days of exposure to 1.25 mg/L of the herbicide. The LC50 values at 48 h were 45.95 mg/L for Roundup, and 30.31 mg/L for Roundup + arsenic. AChE is a neural enzyme that catalyzes the hydrolysis of the neurotransmitter acetylcholine in the nervous system of animals. Chronic exposure to Roundup did not affect AChE activity alone or when mixed with arsenic. Acute exposure to 10 mg/L of Roundup increased AChE activity of *R. arenarum* tadpoles. The CbE enzyme plays an important role in detoxification. CbE activity increased following 48-h exposure to the Roundup but was not affect by chronic exposure to Roundup. GST is part of the first line of cellular defense, and is present in the cytosol of cells involved in the transport and elimination of reactive compounds in aquatic organisms. GST activity increased after 48-hour exposure to Roundup, arsenic, and the mixture of both. However, chronic exposure to Roundup alone or mixed with arsenic did not affect GST activity. Thyroid hormones are essential for tadpole metamorphosis. Roundup alone had no effect on thyroid hormone levels in either the acute or chronic study. Roundup did not induce DNA damage or alter red blood cells.

Wilkens, A. L. L., A. A. N. Valgas, and G. T. Oliveira. 2019. Effects of ecologically relevant concentrations of Boral (R) 500 SC, Glifosato (R) Biocarb, and a blend of both herbicides on markers of metabolism, stress, and nutritional condition factors in bullfrog tadpoles. Environmental Science and Pollution Research 26:23242-23256.

This study compared the effects of a glyphosate-based herbicide (Glifosato) and a sulfentrazone-based herbicide (Boral) on Bullfrog (*Rana catesbeiana)* tadpole metabolism, oxidative stress, and nutritional condition following 7 days of exposure of each herbicide alone or as a mixture. The glyphosate concentration used in this study was 234 µg/L. The glyphosate-based herbicide applied alone had no effect on nutritional condition. Triglycerides and cholesterol increased following glyphosate exposure. Plasma protein, glucose, and uric acid were not affected by glyphosate. In amphibians, corticosterone regulates the metamorphosis induction. Following exposure to glyphosate bullfrog corticosterone production was reduced. Four biomarkers were used to asses oxidative stress (SOD, CAT, TABRS and GST). Of these, only GST was affected by glyphosate.

FISH

Bernal-Rey, D. L., C. G. Cantera, M. dos Santos Afonso, and R. J. Menendez-Helman. 2020. Seasonal variations in the dose-response relationship of acetylcholinesterase activity in freshwater fish exposed to chlorpyrifos and glyphosate. Ecotoxicology and Environmental Safety 187.

The effect of glyphosate exposure on survival and AChE activity in *Cnesterodon decemmaculatus* fish was compared across seasons. Glyphosate had no effect on fish mortality. AChE was inhibited by exposure to 1 and 10 mg/l of glyphosate in all 4 seasons (winter, spring, summer, and fall).

Fabian Bonifacio, A., and A. Cecilia Hued. 2019. Single and joint effects of chronic exposure to chlorpyrifos and glyphosate based pesticides on structural biomarkers in Cnesterodon decemmaculatus. Chemosphere 236.

Bonifacio et al. (2019) measured nuclear abnormalities and liver abnormalities in of *Cnesterodon decemmaculatus* fish that experienced 42-days of exposure to 0.2 and 2 mg/L of Roundup Max. Alterations to liver tissues are particularly relevant due to the liver's role in detoxification of environmental pollutants. The glyphosate-based herbicide had no effect on genotoxicity or liver histology.

Chaulet, F. d. C., H. H. de Alcantara Barcellos, D. Fior, A. Pompermaier, G. Koakoski, J. G. Santos da Rosa, M. Fagundes, and L. J. Gil Barcellos. 2019. Glyphosate- and Fipronil-Based Agrochemicals and Their Mixtures Change Zebrafish Behavior. Archives of Environmental Contamination and Toxicology 77:443-451.

Chaulet et al. (2019) studied how glyphosate influences the behavior of zebrafish. They measured how much time zebrafish spend in the top and bottom of the tank, how long it takes to travel from the bottom to the top, total distance traveled, and the amount of rotating, and crossing. The found that glyphosate had no effect on the time it took to move into the top part of the tank, the frequency of crossing the tank, or the total distance traveled, but it did cause the fish to rotate more frequently and to spend more time at the top of the tank and less time at the bottom. Implications from this study are that fish could be more vulnerable to predators if spending more time nearer to the surface.

Golovanova, I. L., V. K. Golovanov, G. M. Chuiko, V. A. Podgornaya, and A. I. Aminov. 2019. Effects of Roundup Herbicide at Low Concentration and of Thermal Stress on Physiological and Biochemical Parameters in Amur Sleeper Perccottus glenii Dybowski Juveniles. Inland Water Biology 12:462-469.

Golovanova et al. evaluated the effect of 30-day exposure to 2 ug/L of Roundup on body size, intestinal enzyme activity (maltase and amolytic activity), water solubel protein content, and AChE activity in Amur sleeper fish (*Perccottus glenii*). Roundup reduced amolytic activity and water soluble protein content, but had no effect on body size, maltase or AchE activity.

Meshkini, S., M. Rahimi-Arnaei, and A. A. Tafi. 2019. The acute and chronic effect of Roundup herbicide on histopathology and enzymatic antioxidant system of Oncorhynchus mykiss. International Journal of Environmental Science and Technology 16:6847-6856.

Meshkini et al. (2019) assessed the effects of 28 days of Roundup exposure on rainbow trout (*Oncorhynchus mykiss*) by measuring biochemical factors (AChE and liver catalase) and histopathological studies (gills, liver, and kidney tissues). Fish were exposed for 28 days to either 1/5 or 1/10 of the LC50 concentration of Roundup and compared to a control group. Cerebral acetylcholinesterase (AChE) and liver catalase activities decreased following glyphosate exposure at both concentrations. No statistical analyses were used to evaluate structural abnormalities in gill, kidney, or liver tissues and can therefore not be interpreted.

Samanta, P., S. Pal, A. K. Mukherjees, T. Senapati, J. Jung, and A. R. Ghosh. 2019. Assessment of adverse impacts of glyphosate-based herbicide, Excel Mera 71 by integrating multi-level biomarker responses in fishes. International Journal of Environmental Science and Technology 16:6291-6300.

This is a large study that evaluated several biomarkers in 3 different fish species exposed to glyphosate in lab and field conditions. Samanta et al. (2019) found that often times lab conditions resulted on different outcomes than in the field and the physiological response of the fish sto the glyphosate-based herbicide varied between species. The findings collectively suggest that interpretation of lab studies may not be indicidive of risk in real world exposure sceanrios.

REPTILES

Luz Hirano, L. Q., L. d. S. Alves, L. T. Menezes-Reis, J. d. S. Mendonca, K. Simoes, A. L. Quagliatto Santos, and L. G. Vieira. 2019. Effects of egg exposure to atrazine and/or glyphosate on bone development in Podocnemis unifilis (Testudines, Podocnemididae). Ecotoxicology and Environmental Safety 182.

Lus Hirano et al. (2019) studied the 30 and 50 day exposure effects of 65 and 6500 ug/L of glyphosate on bone and cartilage malformations of turtle (*Podocnemis unifilis*) embryos. Glyphosate did not influence the presence or severity of bone or catildege malofmations or other skeletal abnormalities.

BEES

Blot, N., L. Veillat, R. Rouze, and H. Delatte. 2019. Glyphosate, but not its metabolite AMPA, alters the honeybee gut microbiota. Plos One 14.

Blot et al. (2019) evaluated the effect of 15 days of chronic glyphosate or AMPA exposure on the relative abundance of the dominant taxa in honeybee gut microbiota. They further evaluated how chronic exposure of glyphosate and AMPA influenced parasite infection on honeybees. Glyphosate, but not AMPA, decreased the abundance of two of the dominant taxa in honeybee guts and increased the abundance of *Lactobacillus* spp. Neither glyphosate or AMPA had any effect on honey bee survival, food consumption, or *Nosema ceranae* parasite infection. Findings from this study are consistent with Motta et al. (2018) and provides compelling evidence that the gut microbiome of honeybees is influenced by glyphosate, but that it is seemingly inconsequential to honeybee survival.

Decio, P., P. Ustaoglu, T. C. Roat, O. Malaspina, J.-M. Devaud, R. Stoger, and M. Soller. 2019. Acute thiamethoxam toxicity in honeybees is not enhanced by common fungicide and herbicide and lacks stress-induced changes in mRNA splicing. Scientific Reports 9.

It is well understood that neonicotinoids have detrimental impacts on honeybee health and viability. Decio et al. (2019) evaluated whether the combined effects of a neonicotinoid (thiametoxam) and glyphosate were worse than the effects of the neonicotinoid alone. They injected worker bees with the neonicotinoid with and without added glyphosate and measured bee survival and several splicing patterns associated with important genes (x box, elav, and dscam)that are involved in responding to stress. Neither honey bee survival or gene-splicing patterns were affected by the addition of glyphosate. It should be noted that glyphosate concentrations injected into the honey bees was the highest water-soluble concentration possible (23mM) and it was administered at this rate twice over a 48-hour period.

Farina, W. M., M. S. Balbuena, L. T. Herbert, C. Mengoni Gonalons, and D. E. Vazquez. 2019. Effects of the Herbicide Glyphosate on Honey Bee Sensory and Cognitive Abilities: Individual Impairments with Implications for the Hive. Insects 10.

This is a review paper that describes earlier studies that evaluated the effects of glyphosate on foraging behavior of honeybees.

Tome, H. V. V., D. R. Schmehl, A. E. Wedde, R. S. M. Godoy, S. V. Ravaiano, R. N. C. Guedes, G. F. Martins, and J. D. Ellis. 2020. Frequently encountered pesticides can cause multiple disorders in developing worker honey bees. Environmental Pollution 256.

Honey bee larvae were fed an artificial diet that contained glyphosate for six days. They glyphosate concentrations used in this study were the worse-case environmental concentrations previously recorded in pollen or wax from managed honey bee colonies. Glyphosate had no effect on larvae survival, pupae survival, development time, body mass or hypopharyngeal or antenna morphology. Adult honey bees did not up or down regulate any of the 8 measured detoxification genes. Bee larva downregulated one of the 8 genes.

Ulziibayar, D., and C. Jung. 2019. Comparison of Acute Toxicity of Different Groups of Pesticides to Honey Bee Workers (Apis Mellifera L.). Journal of Apiculture 34:305-313.

To understand how glyphosate impacts the survival of a native bee (*Melipona scutellaris)* in Brazil, Uliziibayer and Jung (2019) exposed bees topically and orally to 1/2 the manufactures recommended dose, the manufactures recommended dose, and 2x the manufactures recommended dose. None of these applications impacted bee survival.

TERRESTRIAL INVERTEBRATES

Crone, E. E., E. M. Pelton, L. M. Brown, C. C. Thomas, and C. B. Schultz. 2019. Why are monarch butterflies declining in the West? Understanding the importance of multiple correlated drivers. Ecological Applications 29.

Crone et al. (2019) used two separate modeling approaches to evaluate how factors associated with climate and land use change are contributing to western monarch butterfly abundance.

Monarch abundance was negatively correlated with costal development, glyphosate use, neonicotinoid use, breeding season temperature and breeding season drought. Trends in statewide glyphosate use and coastal development are so highly correlated (r = 0.91) that teasing apart the effects of development and glyphosate use is impossible. The authors conclude that more experimental research is needed to understand the relationship between glyphosate use and Monarch abundance.

Galin, R. R., I. F. Akhtyamova, and E. I. Pastukhova. 2019. Effect of Herbicide Glyphosate on Drosophila melanogaster Fertility and Lifespan. Bulletin of Experimental Biology and Medicine 167:663-666.

Galin et al. (2019) fed fruit flies (*Drosophila melanogaster*) 0.028, 0.28, or 2.8 mg/ml of glyphosate to evaluate the effects of the herbicide on fruit fly fertility and lifespan. The highest concentration (2.8 mg/ml) of glyphosate tested reduced the lifespan of male flies and the number of pupae and imago in the progeny, while the concentrations of 0.028 and 0.28 mg/ml produced no adverse effects. It should be notes that the highest concentration used in this study was the manufacturers recommended concentration for use in the environment.

Hagner, M., J. Mikola, I. Saloniemi, K. Saikkonen, and M. Helander. 2019. Effects of a glyphosate-based herbicide on soil animal trophic groups and associated ecosystem functioning in a northern agricultural field. Scientific Reports 9.

Hagner et al. (2019) hypothesized that in northern latitudes the potential effects of glyphosate-based herbicides on non-target soil organisms could be larger than at lower latitudes because of the shorter growing season. With decreased time to degrade glyphosate, the herbicide could potentially have stronger impacts on soil organisms. They compared the effects of controlling weeds by hoeing, and hoeing + Roundup, to an untreated control. They found that killing plants by hoeing had much larger effects on soil fauna than glyphosate did. The effects of glyphosate were minor and transient and no glyphosate remains were found in the soil at the end of the experiment.

Margus, A., M. Rainio, and L. Lindstrom. 2019. Can Indirect Herbicide Exposure Modify the Response of the Colorado Potato Beetle to an Organophosphate Insecticide? Journal of Economic Entomology 112:2316-2323.

Margus et al. (2019) studied the interacting effects of glyphosate and an insecticide (azinphos-methyl) on Colorado potato beetle (*Leptinotarsa decemlineata*) larval survival and biomarkers associated with stress. Beetle larvae were exposed to potato plants that were grown in soil that had been treated with Roundup and to control plants (grown in soil not treated with Roundup). Beetle larval survival was not affected by Roundup and the Roundup did not influence how the insecticide affected the beetle (i.e. no interaction effect). Glyphosate inhibited the Ldace1 gene expression which encodes AChE activity and increased the activity of a biomarker that is associated with oxidative stress.

Niedobova, J., M. Skalsky, J. Ourednickova, R. Michalko, and A. Bartoskova. 2019. Synergistic effects of glyphosate formulation herbicide and tank-mixing adjuvants on Pardosa spiders. Environmental Pollution 249:338-344.

This study compared the effects of Roundup alone, to Roundup mixed with the surfactants agrovital or wetcit, and the surfactants alone on Pardosa spider predator behavior. Pure surfactants as well as herbicide-and-surfactants tank mixes decreased the predatory activity of Pardosa spiders. Roundup by itself had no effect on spider behavior.

Pochron, S., M. Choudhury, R. Gomez, S. Hussaini, K. Illuzzi, M. Mann, M. Mezic, J. Nikakis, and C. Tucker. 2019. Temperature and body mass drive earthworm (Eisenia fetida) sensitivity to a popular glyphosate-based herbicide. Applied Soil Ecology 139:32-39.

Numerous studied have found conflicting results regarding the effects of glyphosate-based herbicides on earthworms. Pochron et al. (2019) evaluated how initial earthworm body size and soil temperature influence the effect of Roundup on earthworm growth and survival. They found that in hot soil, large worms exposed to Roundup grew larger than large worms not exposed to Roundup (controls), but that growth of large worms in cold soil was not affected by Roundup. The growth of small worms was not affected by Roundup in either hot or cold soil. Worms in cold soil that were exposed to Roundup had lower survival than controls, but the roundup had no effect in hot soil.

Talyn, B., R. Lemon, M. Badoella, D. Melchiorre, M. Villalobos, R. Elias, K. Muller, M. Santos, and E. Melchiorre. 2019. Roundup (R), but Not Roundup-Ready (R) Corn, Increases Mortality of Drosophila melanogaster. Toxics 7.

Talyn et al. (2019) found that there was no difference in life span or reproductive behavior of fruit flies, *Drosphila melanogaster*, that were fed organic corn, non-GMO corn, GMO Roundup resistant corn, and GMO Roundup resistant corn that was sprayed with Roundup. The also determined that the Roundup LC_{50} for male fruit flies was 7.1 and 11.4 g/L for females. The concentrations of glyphosate residues that they detected in the glyphosate treated corn was seven orders of magnitude lower than the LC_{50} for male fruit flys.

AQUATIC INVERTEBRATES

Canosa, I. S., M. Zanitti, N. Lonne, D. A. Medesani, L. S. Lopez Greco, and E. M. Rodriguez. 2019. Imbalances in the male reproductive function of the estuarine crab Neohelice granulata, caused by glyphosate. Ecotoxicology and Environmental Safety 182.

Canosa et al. (2019) evaluated the effects of pure glyposate and Roundup Ultramax on body weight, muscle protein, sperm number and sperm viability of the estuarine crab (*Neohelice granulata*) following 30 days of exposure to the herbicides. The crabs exposed to the herbicides were smaller compared to the control group and had less muscle protein. Roundup, but not pure glyphosate, reduced spem count and inhibited the secreation of hormone from the the androgenic glad.

de Melo, M. S., E. M. Nazari, C. Joaquim-Justo, Y. M. Rauh Muller, and E. Gismondi. 2019. Effects of low glyphosate-based herbicide concentrations on endocrine-related gene expression in the decapoda Macrobrachium potiuna. Environmental Science and Pollution Research 26:21535-21545.

De Melo et al. (2019) studied the effects of chronic (7 and 14 day) exposure to a glyphosate-based herbicide on the expression of genes that are involved in molting and reproduction of crustaceans. They measured the relative transcript expression levels of the ecdysteroid receptor (EcR), the molt-inhibiting hormone (MIH), and the vitellogenin (Vg) genes. Male shrimp upregulated EcR genes after 7 hours of exposure to the 0.65 mg/L dose, but not the higher or lower herbicide concentrations. The lack of a dose response pattern makes it difficult to infer implications. Expression of the EcR gene in females was unaffected by glyphosate following 7 and 14 days of exposure. Vt transcription was upregulated in males following 14 days of glyphosate exposure of the intermediate concentration, but not with lower or higher herbicide concentrations. There was no effect of glyphosate-herbicide exposure on Vg expression of female shrimp. MIH also increased following 7-day glyphosate exposure for the intermediate concentration, but not the higher concentration, and not following 14-days of exposure.

Marineau, E. D., M. J. Perryman, S. P. Lawler, R. K. Hartman, and P. D. Pratt. 2019. Management of Invasive Water Hyacinth as Both a Nuisance Weed and Invertebrate Habitat. San Francisco Estuary & Watershed Science 17:4-4.

Marinaeu et al. (2019) studied the effects of glyphosate applications used to control water hyacinth in the San Joaquin River Delta on invertebrate abundance and diversity. There was no difference in invertebrate abundance or diversity in pre and one-month post glyphosate applications. The authors concluded that current glyphosate use to control water hyacinth poses minimal risk to aquatic invertebrates.

Pala, A. 2019. The effect of a glyphosate-based herbicide on acetylcholinesterase (AChE) activity, oxidative stress, and antioxidant status in freshwater amphipod: Gammarus pulex (Crustacean). Environmental Science and Pollution Research 26:36869-36877.

Pala et al. (2019) evaluated the toxic and sub lethal effects of glyphosate on *Gammarus pulex,* a freshwater amphipod. They measured acetylcholinesterase (AChE) enzyme inhibition in the nervous system, and looked for depletion of antioxidants and the accumulation of reactive oxygen species to determine if the organisms experienced oxidative stress following glyphosate exposure. The AChE is an important enzyme used as a biomarker in biomonitoring studies related to the exposure of pesticides and glyphosate is classified as a non-AChE inhibitor to animals. Pala et al (2019) found that the AChE activity in the freshwater amphipod *Gammarus pulex* was inhibited by 23 to 53% when exposed to 10-40 ug/L of a glyphosate containing herbicide. They also found that the herbicide caused an increase in oxidative stress.

BIRDS

Fathi, M. A., E. Abdelghani, D. Shen, X. Ren, P. Dai, Z. Li, Q. Tang, Y. Li, and C. Li. 2019. Effect of in ovo glyphosate injection on embryonic development, serum biochemistry, antioxidant status and histopathological changes in newly hatched chicks. Journal of Animal Physiology and Animal Nutrition 103:1776-1784.

This study aimed to characterize the impacts of Roundup and pure glyphosate (10 mg/Kg egg) on hatchability, blood parameters, and liver and kidney function after being injected with the herbicides. Roundup reduced the proportion of chicks that hatched by 66%. There was no difference in yolk sac weights or the weight of most of the organs. Glyphosate and Roundup increased liver and kidney mass. In the liver neither GSH or SOD were affected by Roundup or pure glyphosate. Liver MDA increased with both glyphosate and Roundup relative to the controls. In the kidney, GSH was also unaffected by glyphosate and Roundup. Glyphosate and Roundup caused the liver SOD to decreased by the same amount. Glyphosate increased liver MDA while Roundup had no effect on MDA . Glyphosate decreased SOD activity, but Roundup had no effect. Glyphosate and Roundup decreased CAT activity to the same extent.

Rivers, J. W., J. Verschuyl, C. J. Schwarz, A. J. Kroll, and M. G. Bett. 2019. No evidence of a demographic response to experimental herbicide treatments by the White-crowned Sparrow, an early successional forest songbird. Condor 121.

Rivers et al. (2019) used a large-scale experiment to test how nest and post-fledging survival were influenced by herbicide intensity within managed conifer plantations across 2 breeding seasons. They herbicide treatments that were light, moderate, and intensive, and no-spray control. Rivers et al evaluated the reproductive response of the White-crowned Sparrow (*Zonotrichia leucophrys*), a declining songbird in managed forest landscapes of the Pacific Northwest following the herbicide treatments. They found no evidence that either daily nest survival or post-fledging survival were influenced by herbicide application intensity. They also found that vegetative cover at nest sites did not differ across herbicide treatments.

MAMMALS

Bote, K., J. Poeppe, S. Riede, G. Breves, and U. Roesler. 2019. Effect of a Glyphosate-Containing Herbicide on Escherichia coli and Salmonella Ser. Typhimurium in an In Vitro Rumen Simulation System. European Journal of Microbiology and Immunology 9:94-99.

Bote et al. (2019) used an in vitro fermentation study approach to evaluate if glyphosate contaminated food can increase pathogenic bacteria (*E. coli* and *Salmonella*) associated with livestock. Considering that the pathway that is inhibited by glyphosate in plants, is also found in bacteria, it is conceivable that glyphosate may alter bacterial communities in the guts of animals. However, Bote et al found that at even the highest concentrations glyphosate (10 mg/L) had no effect on the abundance of either *E. coli* or *Salmonella*, and had did not promt any resistance to glyphosate or to antibiotics.

SOIL MICROBES

Bruckner, A., A. Schmerbauch, L. Ruess, F. Heigl, and J. Zaller. 2019. Foliar Roundup application has minor effects on the compositional and functional diversity of soil microorganisms in a short-term greenhouse experiment. Ecotoxicology and Environmental Safety 174:506-513.

White clover *(Trifolium repens*) was grown in pots with earthworms and arbuscular mycorrhizal fungi. Glyphosate was applied to the plants and after two weeks microbial respiration was increased by 30%. Glyphosate had a small effect on microbial community composition, decreased bacteria, and had no effect on mycorrhizal fungi. Unfortunately, the experiment did not include a treatment where the plants were killed by cutting without Roundup. It is not possible to distinguish between the effects of Roundup per se and root death. Glyphosate and AMPA were detected in soil leachate, even though it had only been applied to the plants. This suggests that glyphosate entered the soil via the plants.

Farthing, T. S., J. P. Muir, and J. A. Brady. 2020. Three Bermudagrass-suppression techniques have little effect on soil-nutrient availability and microbial communities 200 days after application. Applied Soil Ecology 145.

Farthing et al. (2019) evaluated how different treatments used to remove Bermuda grass (*Cynodon dactylon*) influenced soil microbial communities. They compared repeated glyphosate treatments, and repeated mechanical treatments to each other and to a control. They found that the herbicide treatments had no effect on soil bacteria or archaea species richness or diversity.

Suleman, M., B. J. Keely, S. Liaqat, and W. Ali. 2020. Assessment of the impact of pesticides on the soil microbial community using intact polar membrane lipids as biomarkers. Journal of Animal and Plant Sciences 30:192-204.

Suleman et al. (2019) compared the abundance of 7 polar membrane lipids found in soil spiked with different pesticides, including glyphosate. The lipids are proxies for microbial diversity. They found that of the 7 lipids identified in the soils, glyphosate applied at the maximum rate (1.25 kg/ha) decreased the concentration of 2 lipids, increased the concentration of 1 lipid, and had no effect of 4 of the lipids. The lipid that was found in the highest abundance in the soils is associated with fungi and algae and it decreased by a 10% as a result of the glyphosate addition. It is unclear from this study how changes in the abundance of these lipids influences soil processes such as decomposition rates.

AQUATIC MICROORGAMISMS

Janssen, R., W. Skeff, J. Werner, M. A. Wirth, B. Kreikemeyer, D. Schulz-Bull, and M. Labrenz. 2019. A Glyphosate Pulse to Brackish Long-Term Microcosms Has a Greater Impact on the Microbial Diversity and Abundance of Planktonic Than of Biofilm Assemblages. Frontiers in Marine Science 6

Janssen et al. (2019) evaluated how glyphosate applied to brackish water influenced microbial community diversity and composition (plankton and biofilm forming bacteria). They further evaluated the persistence of glyphosate in the water following application. The bacteria that make up the biofilm can use the phosphorus in glyphosate as a food source. Following the glyphosate addition microbial cell counts initially increased and the then decreased as the glyphosate was consumed by the microbes. After 71 days the glyphosate was reduced by 99%. Glyphosate temporality increased the diversity and richness of the plankton and did not influence the diversity or richness of the biofilm.

Lam, C. H., T. Kurobe, P. W. Lehman, M. Berg, B. G. Hammock, M. E. Stillway, P. K. Pandey, and S. J. Teh. 2020. Toxicity of herbicides to cyanobacteria and phytoplankton species of the San Francisco Estuary and Sacramento-San Joaquin River Delta, California, USA. Journal of Environmental Science and Health Part a-Toxic/Hazardous Substances & Environmental Engineering 55:107-118.

Lam et al. (2019) evaluated the effect of aquatic herbicides, including glyphosate, on the growth of phytoplankton and cyanobacteria that occur in the San Francisco Bay Delta. Of the 3 herbicides tested, glyphosate was the least toxic. At extremely high concentrations (7 x 10^4 ug/L) glyphosate inhibited growth of 2 plankton species, but increased growth of the other. At environmentally relevant concentrations glyphosate had no effect on phytoplankton or cyanobacteria growth.

PLANTS

de Moraes, C. P., I. P. F. S. de Brito, L. Tropaldi, C. A. Carbonari, and E. D. Velini. 2019. Hormetic effect of glyphosate on Urochloa decumbens plants. Journal of Environmental Science and Health Part B-Pesticides Food Contaminants and Agricultural Wastes.

de Moraes et al. (2019) used greenhouse experiments to evaluate how a non-target plant, *Urochloa decumbens*, responded to different concentrations of glyphosate. They found that at low concentrations glyphosate had no effect on plant growth, but that high concentrations (>11 g/ha) glyphosate decreased plant growth.

Enloe, S. F., K. H. Quincy, M. D. Netherland, and D. K. Lauer. 2020. Evaluation of fluazifop-P-butyl for para grass and torpedograss control in aquatic and wetland sites. Journal of Aquatic Plant Management 58:36-40.

Enloe and Netherland planted native aquatic plants along with the torpedo grass (*Panicum repens*) into a mesocosm experiment and applied glyphosate and imazapyr to evaluate how the herbicides influenced the native species. They further evaluated if the impacts on native plants varied in the spring and fall. Both glyphosate and imazapyr reduced native plant biomass by 64-100%. Season of application did not influence the extent that the herbicides reduced native plant biomass. The authors do not explain whether the herbicide was intentionally applied onto the native plants or if it was only applied to the invader. The herbicide reduced mycorrhizal biomass relative to a control, but had the same effect as the other removal treatments.

Helander, M., A. Pauna, K. Saikkonen, and I. Saloniemi. 2019. Glyphosate residues in soil affect crop plant germination and growth. Scientific Reports 9.

Helander et al. (2019) used a greenhouse and a field experiment to evaluate how Roundup and pure glyphosate effect seed germination and growth of four crop plants. In the greenhouse, soils were treated with Roundup or glyphosate and after 14 days the crop plants were planted into the treated soils. In the field, Helander et al treated soils in agricultural fields with Roundup and planted crops 3 weeks later. Fava bean seed germination was not affected by the herbicide treatments in the greenhouse experiment, but the seeds germinated faster in the untreated controls. The plants grown in the control soils were larger than in the Roundup treated soils, Crop yield was unaffected by the herbicides. In the greenhouse, oats germinated faster in the control soil than in the herbicide treated soils. Oat plants in the Roundup treated soil were smaller that in plants in the untreated control soil, but not the glyphosate soil. In the field, the oats were damaged by geese herbivory, but the lack of a significant treatment x herbivory damage interaction term suggests that geese did not prefer one soil treatment over the others. In the field, oats started out larger in the control, but by the end of the study were the same size in the control and herbicide treated soils. No traces of glyphosate or AMPA were detected in the greenhouse soils.

Wood, L. J. 2019. The presence of glyphosate in forest plants with different life strategies one year after application. Canadian Journal of Forest Research 49:586-594.

Wood (2019) measured glyphosate in the roots, shoots, and fruits of native traditional-use plants with different life strategies (i.e. herbaceous, woody). Perennial herbaceous and woody plants were collected one year after forestry-based applications of glyphosate. Glyphosate residues were found one-year after application. The highest and most consistent levels of glyphosate and AMPA were found in herbaceous perennial root tissues, but shoot tissues and fruit were also shown to contain glyphosate in some species. Findings from this study indicate that glyphosate cam be stored in root structures of perennial plants during dormancy periods and move up to shoot and fruit portions in years following applications in some species.

ENVIORNMENTAL FATE

Muskus, A. M., M. Krauss, A. Miltner, U. Hamer, and K. M. Nowak. 2019. Effect of temperature, pH and total organic carbon variations on microbial turnover of (13)CA(3)(15)N-glyphosate in agricultural soil. Science of the Total Environment 658:697-707.

The degradation of glyphosate in soil may vary with different environmental conditions. Muskus et al. (2019) evaluated the effects of soil temperature, total organic carbon, and soil pH on the rate of microbial conversion of glyphosate to CO_2. Higher temperature and total organic carbon content increased microbial conversion of glyphosate to CO_2. The influence of the soil pH or total organic carbon treatment on the extent of glyphosate mineralization was more discernible at lower temperatures. This study highlights how the rate of glyphosate degradation depends upon many factors including temperature, soil pH, and soil carbon content.

Gu, X., Y. Cen, L. Guo, C. Li, H. Yuan, Z. Xu, and G. Jiang. 2019. Responses of weed community, soil nutrients, and microbes to different weed management practices in a fallow field in Northern China. Peerj 7.

Gu et al. (2019) evaluated how different invasice species management treatments influence invader seed banks, soil nutrients, and soil microbial biomass. They compared the effects of physical removal treatments (tilling) with Roundup treatments. The physical removal treatment reduced weed seeds by 34%, while the herbicide treatment did not reduce weed seeds. Roundup treatments had smaller effects on total microbial biomass than the physical treatments.

1.2 IMAZAPYR

No studies were published in 2019 that describe human health risks or the environmental fate of imazapyr.

NON-TARGET IMPACTS

McCaskill, G. L., S. Jose, and A. V. Ogram. 2019. Low-dose Herbicide Effects on Tree Establishment and Soil Nitrogen Biogeochemistry within Pine Savannas. Soil Science Society of America Journal 83:S153-S160.

McCaskill et al. (2019) compared the effects of imazapyr (applied at at rate of 0.21 kg/ha) on soil nitrogen mineratlization, soil microbial biomass, and the survivl and growth of long leaf pine. Imazapyr was the only treatment to significantly improve growth over the control in a single application. The herbicide increased N mineralization rates, and ammonification. Microbial biomass was not affected by a single imazapyr application.

EFFICACY

Annen, C. A., J. A. Bland, A. J. Budyak, and C. D. Knief. 2019. Effects of Selectively-targeted Imazapyr Applications on Typha angustifolia in a Species-rich Wetland (Wisconsin). Ecological Restoration 37:3-4.

In this simple and well written short format paper Annen et al. (2019) evaluated multiple apporaches to manage narrow leaved cattail (*Typha angustifolia*) in areas where it does not grow in a monoculture. They compared imazapyr and mowing treatments to an untreated control. The applied imazapyr twice and after 2 years the herbicide plots had more plant diversity than the control, put purple loosestrife *(Lythrium salicaria*) increased in abundance following Typha control.

Berrill, J.-P., and R. Howe. 2019. Multiaged redwood responds well to partial harvest and herbicide treatments. Canadian Journal of Forest Research 49:1425-1433.

Berrill and Howe (2019) studied coast redwood growth following imazapyr herbicide treatments used to remove tan oak competition. Removal of tan oak with the herbicide increased redwwod radial growth in 23% of the redwood trees measured, while 67% of the untreated control trees had a reduction in basal area. The ratio 8 years post treatment to 8 years pre treatment was 59% higher basal area in herbicide only pots, and 108% higher when herbicide was combined with selective harvests of redwoods than untreated control plots

Goodall, J., and M. Braack. 2019. Screening herbicides for the control of the wetland invader, Sambucus nigra L., in South Africa. African Journal of Aquatic Science 44:295-299.

Goodall and Braack et al (2019) tested the efficcaicy of 5 different herbicides used to treat *Sambucus nigra*. Two of the 9 herbicides in the study were triclopyr and imazapyr. Applied to cut stumps both imazapyr and triclopyr at the lowest recomneded application rate resulted in greater than 80% mortlality.

Murray, L., B. J. Schutte, C. Sutherland, L. Beck, A. Ganguli, and E. Lehnhoff. 2019. Integrating conventional management methods with biological control for enhanced Tamarix management. Invasive Plant Science and Management 12:176-185.

Murray et al. (2019) studied how Tamarix responds to foliar imazapyr applications in the presence of the Tamarix leaf beetle biocontol agent. They found that at the standard application rate (3.6 g/L) of imazapyr improved Tamarix control when applied in conjuction with the presence or the biocontrol.

Rohal, C. B., C. Cranney, E. L. G. Hazelton, and K. M. Kettenring. 2019. Invasive Phragmites australis management outcomes and native plant recovery are context dependent. Ecology and Evolution 9:13835-13849.

Rohal et al. (2019) compared different management treatements to control Phragmites. They compared the efficacy of glyphosate and imazapyr on controling the invader and on native plant recovery. They found the glyphosate and imazapyr worked equally well at controlling phragmites and that fall applications were more effective than summer applications. They expected native plant recruitment to be lower following imazapyr applications compared to glyphosate, because imazapyr has a longer half life and is purported to be able to persist in the soil. Contrary to their predictions, imazapyr did not restrict native plant recruit more than glyphosate. The authors speculate that this may be because the persistence of imazapyr in the anerobic soil conditions associated with wetlands is lower than in aerobicoic soil.

1.3 AMINOPYRALID

No studies were published in 2019 that describe human health risks, ecological effects, or the environmental fate of aminopyralid.

EFFICACY

Dias, J. L. C. S., G. E. Duarte, W. L. Colombo, and B. A. Sellers. 2019. Cadillo (Urena lobata) control with POST herbicides. Weed Technology 33:387-392.

Dias et al. (2019) compared the efficacy of 7 different herbicides for control of *Urena lobata* (cadillo) in cattle pastures. Triclopyr and aminopyralid were the only two herbicides that were tested that are relevant to MROSD. They found that both of these herbicides resulted in >90% target plant mortality.

1.4 CLOPYRALID

No studies were published in 2019 that describe human health risks or the environmental fate of clopyralid.

NON-TARGET EFFECTS

MacDonald, N. W., K. M. Dykstra, and L. M. Martin. 2019. Restoration of native-dominated plant communities on a Centaurea stoebe-infested site. Applied Vegetation Science 22:300-316.

MacDonald et al. (2019) used a myriad of restoration approaches to increase cover of native forbs and grass in an area dominated by *Centaurea stoebe* (spotted knapweed). To remove *C. stoebe* prior to seeding with native forbs MacDonald et al sprayed study sites with either clopyralid or glyphosate, or mowed them. They found that clopyralid treated plots, and mowed plots had fewer native species than polts that were treatend with glyphosate.

EFFICACY

Farooq, M. H., Q. U. Zaman, N. S. Boyd, and S. N. White. 2019. Evaluation of broadcast and spot herbicide applications for narrowleaf goldenrod Euthamia graminifolia (L.) Nutt. management in lowbush blueberry. Weed Technology 33:739-747.

Farooq et al. (2019) compared the efficacy of spraying goldenrod that occurs in blueberry crops with a number of different herbicidds including glyphosate (7.24 g/L), clopyralid (0.08 g/L) and tricolpyr (1.68 g/L). They compared fall and summer spot treatments and found that summer applictions of either herbicide reduced shoot density, but that only glyphosate reduced shoot density in the fall.

1.5 TRICLOPYR

ENVIRONMENTAL FATE

Rodrigues, E. T., M. F. Alpendurada, A. Guimaraes, R. Avo, B. Ferreira, and M. A. Pardal. 2019. The environmental condition of an estuarine ecosystem disturbed by pesticides. Environmental Science and Pollution Research 26:24075-24087.

To understand how agricultural applications of pesticides, including triclopyr, persit in estuaries Rodrigues et al (2019) sampled surface water and sediments within an estuary in Portugal before and after the pesticides were applied to crops. Triclopyr was not found in concentrations that exceed the EU's acceptable limits in either the water or sediment samples. The authors conclude that the agrucltural pesticides used in the region pose minimal riskto aquatic orgamism that occur in the studied estuary.

EFFICACY

Frey, M., and J. P. Schmit. 2019. Controlling Italian Arum (Arum italicum). Natural Areas Journal 39:372-377.

Frey et al. (2019) tested the effectiveness of seveal different herbicide formulations on controlling Italian arum (*Arum italicum*) with everal herbicide forimulations. Triclopur was one of the herbicides evaluated. They found that Triclopyr was ineffective at controling the invader and that after 1 year there was no difference in arum abundnce in the triclopyr and control plots.

Gibson, D. J., L. A. Shupert, and X. Liu. 2019. Do No Harm: Efficacy of a Single Herbicide Application to Control an Invasive Shrub While Minimizing Collateral Damage to Native Species. Plants-Basel 8.

Gibson et al. (2019) suggests that collatoral damage to non-taget plants following invasice species control should be considered following restoration actions. They assesed the efficacy pf various tricolyp formualtions on controling *Lespedeza cuneate* and evaluated how the treatments influenced the plant community. Tricolpyr reduced the abundnca of the invader following initial application, but that it had regained dominance within 3 years. Native species initially declined in abundance following the herbicide treatments but recovered to pretreatment abundances

Marble, S. C., and A. Chandler. 2019. Control of skunk-vine (Paederia foetida L.) with preemergence and postemergence herbicides in central Florida during the winter season. Invasive Plant Science and Management 12:51-59.

Marble et al. (2019) used greenhouse nad field experiments to compare the efficacy of several different herbicides on controlling skunk vine (*Paederia foetida*). Aminopyralid, triclopyr, and glyphosate were amoung the herbicides that were evaluated. All three of these herbicides were equally effective at controlling the skunk vine in this experiment.

Prince, C. M., K. H. Quincy, S. F. Enloe, J. Possley, and J. Leary. 2019. Cut-stem treatments using graminicides for burmareed (Neyraudia reynaudiana) invasions in Pine Rocklands, South Florida, USA. Invasive Plant Science and Management 12:236-241.

Prince et al. (2019) compared the efficacy of several herbicides and application approaches on Burmareed (*Neyraudia reynaudiana*)plants grown in the greenhouse and the field. They found that triclopyr and glyphosate applied either immediately after stump cutting or applied a year were equally effective and that glyphosate and tricolpyr provide high level control in the greenhouse study, but were realtively uneffective in the field. Under field conditions the two herbicides provided less than 30% control irrespective of whether the herbicides were applied to cut stumps immediately following cutting or the following year after repsroutin occurred.

1.6 CLETHODIM

NON-TARGET EFFECTS

Xiong, G., L. Zou, Y. Deng, Y. Meng, X. Liao, and H. Lu. 2019. Clethodim exposure induces developmental immunotoxicity and neurobehavioral dysfunction in zebrafish embryos. Fish & Shellfish Immunology 86:549-558.

Xiong et al. (2019) examined the effects of clethodim on the development, immune toxicity, cell death (apotosis), and locomotion on zebrafish larvae. The larvae were exposed to 10, 50, 100, 300 or 500 ug/L of clethodim. All doses reduced larval survival, and doses > 50 ug/L reduced larval size and heart rate. Cell death and oxidative stress increased in a dose dependent fashion. Together these findings provide evidence that chronic exposure to high doses of clethodim can result in lethal and sublethal effects on zebrafish larvae.

ENVIRONMENTAL FATE

Anastassiadou, M., A. Brancato, L. C. Cabrera, L. Ferreira, L. Greco, S. Jarrah, A. Kazocina, R. Leuschner, J. O. Magrans, I. Miron, R. Pedersen, M. Raczyk, H. Reich, S. Ruocco, A. Sacchi, M. Santos, A. Stanek, J. Tarazona, A. Theobald, A. Verani, and E. F. S. European Food Safety Authority. 2019. Review of the existing maximum residue levels for clethodim according to Article 12 of Regulation (EC) No 396/2005. Efsa Journal 17.

Anastssidue et al. (2019) is a review conducted by the Europoean Food Safety Authority that attempted to determine what the maximum residue concentration for clethodim should be in for processed commodities, rotational crops, and livestock. They were unable to identify a maxixum residue level because of a paucity of data regarding risk. They did conclude that there is potential for clethodim to have a genotoxic effect, but that more studies are needed to understand if relevant concentrations pose risks to non-target orgamisms.

EFFICACY

Cassol, M., M. D. Mattiuzzi, A. J. P. Albrecht, L. P. Albrecht, L. C. Baccin, and C. N. Z. Souza. 2019. Efficiency Of Isolated And Associated Herbicides To Control Glyphosate-Resistant Sourgrass. Planta Daninha 37.

Cassol et al. (2019) compared the effectiveness of different herbicide applications to conrtol perrenial sourgrass (*Digitaria insularus*). Two of the treatments included clethodim alone and glyphosate + clethodim. Clethodim and clethodim + glyphosate initially removed 90% of the sourgrass cover, but ater 35 days the plants were resprouting.

2.0 ADJUVENTS/SURFACTANTS:
Alcohol ethoxylate, Alkylphenol ethoxylate, Lecithin, Canola oil: ethyl & methyl esters

No studies were published in 2019 that describe human health risks, ecological effects, or the environmental fate of the adjuvants and surfactants used by MROSD.

3.0 FUNGICIDES

No studies were published in 2019 that describe human health risks, ecological effects, or the environmental fate of the fungicides used by MROSD.

4.0 INSECTICIDES

4.1 DIATOMACEOUS EARTH

No studies were published in 2019 that describe human health risks, ecological effects, or the environmental fate of diatomaceous earth.

4.2 D-TRANS ALLETHRIN

No studies were published in 2019 that describe human health risks, ecological effects, or the environmental fate of *D-trans allethrin*.

4.3 FIPRONIL

Harley, K. G., K. L. Parra, J. Camacho, A. Bradman, J. E. S. Nolan, C. Lessard, K. A. Anderson, C. M. Poutasse, R. P. Scott, G. Lazaro, E. Cardoso, D. Gallardo, and R. B. Gunier. 2019. Determinants of pesticide concentrations in silicone wristbands worn by Latina adolescent girls in a California farmworker community: The COSECHA youth participatory action study. Science of the Total Environment 652:1022-1029.

Harley et al. (2019) assesed weekly pesticide exposre in 14-16 year old latina girls that live in the Salinas Valley, an agricultual region of California. The study participants wore silicon wrist bands for a week that were analyzed for pesicide concentrations. Fipronil was detercted on 87% of the wrist bands and was the most frequntly detercted pesticide. In California, fipronil is not used in agricultue and is only used for treating termites, roaches, and ants in and around buildings, or to treat fleas and ticks on dogs and cats. The authors do not address what pathway the study participants were exposed to fipronil, but did find that living within 100 m of active agricultural fields, having carpeting in the home, and having an exterminator treat the home in the past six months were associated with higher odds of detecting certain pesticides.

Pandit, A. A., R. K. Gandham, C. S. Mukhopadhyay, R. Verma, and R. S. Sethi. 2019. Transcriptome analysis reveals the role of the PCP pathway in fipronil and endotoxin-induced lung damage. Respiratory Research 20.

Pandit et al. (2019) used a microarray and qPCR approach to evaluate the effects of fipronil on the transcriptome profile associated with the lungs of mice that were fed 1/10th and 1/20th of the LC50 for 90 days. They concluded that these concentrations of fipronil can alter the transcriptome profile of lungs and potential cause damage through the production of cytokines that cause lung inflammation.

Seydi, E., L. Mehrpouya, H. Sadeghi, S. Rahimi, and J. Pourahmad. 2019. Toxicity of fipronil on rat heart mitochondria. Toxin Reviews.

Seydi et al. (2019) studied the effects of in vitro fipronil exposure on rat heart mitochondria function. They found that fipronil increases mitochondrial oxidative stress (increased production of ROS and LPO, mitochondrial swelling, and cytochrome C release) following apoptosis signaling. These impacts on mitochondria likely have hazardous effects on heart tissues.

Testa, C., S. Salis, N. Rubattu, P. Roncada, R. Miniero, and G. Brambilla. 2019. Occurrence of Fipronil in residential house dust in the presence and absence of pets: a hint for a

comprehensive toxicological assessment. Journal of Environmental Science and Health Part B-Pesticides Food Contaminants and Agricultural Wastes 54:441-448.

Testa et al. (2019) compared the amount fipronil in dust within homes with and without pets. They found that fipronil dust contamination in the presence of pets was far higher than in homes without pets. The amount of fipronil found in homes with and without pets never exceeded the concentrations considered hazardous by the US (EPA) or EU(ECHA) regulatory agencies. This paper is not directly useful to MROSD because it does not account for the presence or absence of fipronil bait stations in homes, but never the less, it is reassuring that even in homes with pets concentrations of fipronil are considered safe.

4.5 INDOXACARB

Wang, C., A. Eiden, R. Cooper, C. Zha, D. Wang, and E. Reilly. 2019a. Changes in Indoor Insecticide Residue Levels after Adopting an Integrated Pest Management Program to Control German Cockroach Infestations in an Apartment Building. Insects 10.

Wang et al. (2019) measured residues of indoxocarb on kitchen and bedroom floors of 69 low income apartments in New Jersey that used Advion indoxacarb bait traps and insect sticky traps to treat german cockroaches. In each apartment 18 g of gel bait (with 0.6% indoxacarb) was applied every two weeks for as long as cockroaches were still present. Tweleve months following the onset of the study indoxacarb was detectable in only 2 out of 69 apartments.

Vernis, L., H. Zorn, B. Glandorf, L. Herman, J. Aguilera, M. Andryszkiewicz, Y. Liu, A. Chesson, and Efsa. 2019. Updated peer review concerning the risk to mammals and bees for the active substance indoxacarb. Efsa Journal 17.

The European Food and Safety Authority completed at risk assement for the use of indoxocab to control pest in corn crops on small herbiverous and earthworm eating mammals, honeybees, and bumble bees. The committee concluded that the use of idoxacarb in corn crops poses high risk for secondary poisining of small herbiverous mammals following a single application, and to earthworm eating mamamals following repeated applications. They also concluded that indoxocarb adult honeybees and bumble bees risk have a high risk of being poisoned if the indoxacarb is appliesd before or during the flowering period, but not if applied after flowering.

4.6 PHENOTHRIN

No studies published in 2019 described the human health risks, ecological effects, or the environmental fate of phenothrin.

4.7 PRALLETHRIN

No studies published in 2019 described the human health risks, ecological effects, or the environmental fate of prallethrin.

4.8 S-HYDROPRENE

No studies published in 2019 described the human health risks, ecological effects, or the environmental fate of S-Hydroprene.

4.9 SODIUM TETRABORATEDECAHYDRATE

No studies were published in 2019 that describe human health risks, ecological effects, or the environmental fate of sodium tetraborate decahydrate.

5.0 RODENTICIDE

5.1 CHOLECALIFEROL

No studies were published in 2019 that describe human health risks, ecological effects, or the environmental fate of cholecalciferol when it is used as a rodenticide.