Midpeninsula Regional Open Space
Integrated Pest Management Program

2020 Pesticide 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 2020 that describe the effects of pesticides used by Midpeninsula Regional Open Space District (hereafter MROSD) on non-target organisms. Also included is the persistence of pesticides in the environment as well as their effectiveness at controlling invasive species.

1.2 LITERATURE REVIEW AND EVALUATION PROCESS

We performed a comprehensive literature review of all the scientific papers published in 2020 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. We 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 2020. 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 A). 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.

The effects of glyphosate on human health continue to be debated, particularly as they apply to cancer-risk. In 2015, the International Agency for Research on Cancer (IARC), a group of expert scientists assembled by the World Health Organization (WHO), classified glyphosate as “probably carcinogenic to humans”. The IARC’s intention was to evaluate the ability of glyphosate, among other pesticides, to cause cancer. This classification was based primarily on experimental evidence of cancer in lab animals, and less so on evidence of cancer in humans following real-world exposure. Other agencies that have performed glyphosate risk assessments, in other words the probability that glyphosate will cause cancer based on expected exposure levels, have come to different conclusions. As of January 2020, the U.S. Environmental Protection Agency (EPA), a regulatory agency, maintained its classification of glyphosate as “not likely to be carcinogenic to humans”. Several other regulatory agencies around the world have made similar classifications and the IARC remains one of the few groups to classify glyphosate as a “probable human carcinogen”.

In the last few years, several large-scale studies have been published on the association between glyphosate exposure and the risk of non-Hodgkin lymphoma (NHL). Two particularly notable analyses utilized data from the Agricultural Health Study (AHS), which is a long-term cohort study monitoring the health of over 50,000 pesticide applicators from the US. One study, Andreotti et al. (2018), concluded that glyphosate did not increase the risk of NHL. However, Zhang et al. (2019), using the AHS along with a handful of other studies, reported that the risk of NHL was increased in high exposure groups. Leon et al. (2019) also considered data from the AHS, as well as cohort studies from France and Norway, and found that glyphosate exposure generally did not increase the risk of NHL, except for the DLBCL subtype for which risk was slightly increased. In 2020, a meta-analysis was performed by Donato et al. (2020) evaluating the relationship between glyphosate exposure and NHL or multiple myeloma (MM). They included seven relevant cohort and case-control studies, one of which was Leon et al. (2019), to determine the relative risk of NHL or MM. For NHL, there was a relative risk of 1.03 (CI: 0.86-1.21) and for MM it was 1.04 (CI: 0.67-1.41). When interpreting risk ratios, values greater than 1 with a confidence interval that does not overlap with 1 are indicative of a relationship. Given that both NHL and MM have confidence intervals that overlap with 1, there is no indication of an association between glyphosate exposure and elevated risk of NHL or MM. They also reported evidence of publication bias in the literature caused by the failure to report small, non-positive studies. It is worth noting the differences in methodology of these studies. Leon et al. (2019) and Donato et al. (2020) considered glyphosate exposure as never or ever exposed whereas Andreotti et al. (2018) and Zhang et al. (2019) related cancer risk to the extent of glyphosate exposure. Treating glyphosate exposure as a binary variable (never vs. ever exposed) can be limiting, but considering only the highest exposure groups, as Zhang et al. (2019) did, may be misleading.

Although the majority of studies discussed in this report suggest that glyphosate exposure does not increase cancer risk, the general lack of consensus in the scientific literature and the presence of publication bias, reported by Donato et al. (2020), make it difficult to form definitive conclusions regarding glyphosate's link to cancer. **That said, all of these studies taken together suggest that that there is no relationship between glyphosate exposure and non-Hodgkin lymphoma (NHL) or multiple myeloma (MM).** Regardless of how the glyphosate-cancer evidence is interpreted, it is important to note that there are substantial differences in the degree of exposure between agriculture and wildland weed workers. To date, all of the reviewed studies evaluating the carcinogenicity of glyphosate are in the context of agricultural pesticide application, which is characterized by frequent glyphosate use and indiscriminate application methods and pose a considerably greater risk to applicators than the application practices of MROSD. The rates of exposure experienced by agricultural workers generally far exceeds those of wildland weed workers.

In addition to concerns about cancer, glyphosate has also been included in lists of substances/factors suspected to play a role in the development of Autism Spectrum Disorder (ASD/autism). The cause(s) of autism are not fully understood, but a recent rise in the prevalence of ASD has sparked research into possible genetic and environmental factors. The rise of ASD, coupled with the increased use of glyphosate, has led many to investigate the potential connection between the two. Pu et al. (2020) used mice to study the link between maternal glyphosate exposure and ASD. They found that offspring of mothers that had been exposed to 0.098% glyphosate while pregnant showed behavioral changes associated with ASD. Brain biochemical changes, also associated with ASD, were observed as well. Due to our limited understanding of the disorder, the implications of the biochemical changes observed in this study, as they relate to ASD, are speculative. Before a causal link can be made between glyphosate and ASD, more data is needed regarding the causes and development of autism. Importantly, the authors note that “it is exceptionally unlikely that such exposure could be reached during human pregnancy” (Pu et al. 2020).

A handful of studies from 2020 investigated glyphosate levels in the body following exposure. Companion studies performed by Pierce et al. (2020) and Kougias et al. (2020) evaluated urinary glyphosate levels and corresponding internal doses of glyphosate in residential applicators following a heavy exposure scenario. Urinary glyphosate levels were measured in applicators that lacked either respiratory PPE (respirator) or dermal PPE (Tyvek suit) while applying Roundup with a backpack sprayer for 100 minutes. Both groups had elevated urinary glyphosate levels following exposure, but they returned to baseline levels after 24 hours (Pierce et al. 2020). Furthermore, estimations of internal glyphosate doses from these applicators were considerably lower than even the most conservative levels of acceptable daily intake (ADI), suggesting that glyphosate is quickly expelled from the body (Kougias et al. 2020). For example, according to the National Pesticide Information Center the acceptable daily intake for glyphosate is 100 µg/kg and the highest daily values reported by Kougias et al. (2020) did not exceed 6 µg/kg. Even in high exposure, it would appear the health risks are negligible. However, due to the small sample size and preliminary nature of these studies, more research is needed to corroborate these findings. An update of the literature review by Gillezeau et al. (2019) included five new studies regarding urinary glyphosate levels in children and occupationally exposed adults. The conclusions of Gillezeau et al. (2020) were similar to those of Gillezeau et al. (2019); urinary glyphosate concentrations were highly variable and information regarding exposure severity, use of PPE, and urinary glyphosate levels in the general public is lacking. Mueangkhiao et al. (2020) evaluated the relationship between kidney biomarker levels and pesticide exposure in Thai farmers. They measured biomarker levels in urine samples collected from 59 farmers who were interviewed about their lifestyle and pesticide usage. The majority of farmers applied pesticides at least once a week, and although pesticides were always applied in mixtures, glyphosate was the most commonly used. Kidney biomarker levels were within the normal range, but they did find a positive association between glyphosate use or pesticide exposure intensity and serum creatinine, a common kidney function biomarker; increased serum creatinine levels can be a sign of poor kidney function. However, because the authors did not measure internal or urinary pesticides levels of the farmers, it is unclear what the relationship is between glyphosate exposure and kidney function.

A review done by Agostini et al. (2020) provided important insights on the evidence of glyphosate related health risks in scientific literature. Their summary of 144 studies outlining the effects of glyphosate on cells, cancer, respiratory diseases, neurological effects, reproductive effects, and more, concluded that glyphosate has some toxicological effect on human cells in vitro, but these effects are not apparent in epidemiological studies. These studies taken together do not suggest that glyphosate exposure results in serious health issues. However, a minority of the studies do describe health impacts that warrant further investigation.

We 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 a fact sheet (http://npic.orst.edu/factsheets/glyphogen.html).

2.1.2 Non-target effects

In this section, we summarize the studies published in 2020 investigating the impact of glyphosate on non-target animals, microbes, and plants. Although there are many compelling studies outlining the various effects of glyphosate exposure, it is important to consider these effects in the context of invasive plant management. The degree of indirect glyphosate exposure is dependent upon the magnitude and method of application and persistence of the herbicide in the environment. Considering that MROSD typically employs precise application methods, non-target exposure is assumed to be relatively minimal. However, despite best efforts, non-target organisms may still come into contact with glyphosate residues. Battaglin et al. (2014) measured glyphosate levels in the environment and determined the maximum concentration of glyphosate in aquatic and terrestrial habitats to be 301 µg/L and 476 µg/kg, respectively. Here, we consider these concentrations to be environmentally realistic, although they are reflective of a worst-case exposure scenario.

2.1.2.1 Mammals

Due to the heavy use of glyphosate in agriculture, papers outlining the impacts of glyphosate on mammals focus on livestock. Studies from 2020 primarily considered the effects on the reproductive potential of cows, pigs, and lambs. For cows, glyphosate did not affect blood cell functionality, but did compromise preimplantation embryos and, at 0.9 mg/kg, increased the incidence of cell death by roughly 10% (Schnabel et al. 2020 & Cai et al. 2020). It is unclear how glyphosate would affect natural fertilization and embryo development following exposure to realistic concentrations. There is some evidence suggesting that glyphosate exposure can negatively affect pig gametes. Pig sperm had reduced survival and severely reduced mobility following exposure to 360 µg glyphosate/mL of Roundup (Nerozzi et al. 2020). Spinaci et al. (2020) found that pig ovarian cells were also affected by glyphosate, but to a much lesser extent than sperm cells, with reported delays in maturation but no effects on survival. In studies on gut and intestinal parameters, Krause et al. (2020) found that 228 g glyphosate/day had no effect on pig gut microbiota, and Qiu et al. (2020) reported increased antioxidant enzyme activity but no effects on intestinal morphology when piglets were fed glyphosate at a minimum of 20 mg/kg. Lambs experienced some reduction in cell proliferation and gene expression in uterine tissues when injected with 2 mg/kg glyphosate, but there were no changes on uterine morphology (Alarcon et al. 2020). The majority of these studies examined glyphosate exposure in vitro. Livestock are most likely to be exposed to glyphosate via residues in feed. The European Food Safety Authority determined that the maximum allowable daily intake of glyphosate for cattle and pigs is 13.17 mg/kg/day and 2.85 mg/kg/day, respectively (EFSA 2018). However, if livestock are grazing they are unlikely to come into contact with glyphosate concentrations at this magnitude.

2.1.2.2 Birds

Three studies were conducted in 2020 regarding the non-target impacts of glyphosate on birds. Fathi et al. (2020) found that injecting eggs with Roundup at a dose of 10 mg glyphosate/kg increased liver weight, negatively affected intestinal morphology, and altered the activity of antioxidant enzymes in chicken embryos. It is unclear how unhatched birds in the wild would be directly exposed to glyphosate, especially at this magnitude. Ruuskanen et al. (2020a) monitored the health of Japanese quails (*Coturnix japonica*) that were fed glyphosate-contaminated food at a rate of 160 mg/kg. After 52 weeks of exposure, they found some changes in the gut microbiome, but no effects on body mass or reproduction. In a follow-up study on the offspring of these birds, Ruuskanen et al. (2020b) found no evidence to suggest that parental glyphosate exposure has any effects on offspring. Ingestion of glyphosate is a likely route of exposure, as birds may consume fruits or seeds of glyphosate-treated plants. However, the concentrations used here far exceed those reported in the wild, but even so, the effects on individuals and their offspring are minimal.

2.1.2.3 Reptiles

In 2020, one paper investigated the effects of glyphosate on reptiles. Mestre et al. (2020) reported alterations in the immune response, specifically white blood cells, of tegu lizard (*Salvator* *merianae*) embryos after topical exposure to a minimum of 400 µg/egg of glyphosate-based herbicides. In this study, eggs were sprayed directly with herbicides. Most lizards will make efforts to conceal their eggs, either by digging a hole or covering them with debris, so it is unlikely that eggs would come into direct contact with glyphosate in the wild.

2.1.2.4 Bees

In recent years, widespread bee decline has prompted research into the effects of pesticides, particularly glyphosate, on bee health and survival. Direct contact with herbicides, while uncommon for most organisms, is of reasonable concern for bees and other flying insects when and where herbicides are applied. Aerially applications or applications with large machinery will result in greater exposure than targeted applications. However, the probability that bees will be directly exposed to herbicides is greatly reduced by the use of backpack sprayers and similarly precise methods that are frequently used by MROSD. Indirect exposure can occur as a result of foraging on treated plants or consuming pollen or nectar that may contain pesticide residues, but our knowledge of environmentally realistic glyphosate levels in these resources is extremely limited.

Honeybees (*Apis mellifera*) that were exposed to 0.1-10 µg/L of glyphosate for 20 days experienced mortality rates that were 8-15% higher than unexposed bees. Those that survived exposure consumed larger quantities of food, perhaps as a result of stress (Almasri et al. 2020). Conversely, when honeybees were exposed to glyphosate for 120 hours, food consumption and survival were unchanged. However, when dually exposed to glyphosate and *Nosema* sp. (a pathogenic microsporidian), both consumption and survival decreased (Faita et al. 2020). Furthermore, feeding behavior of mason bees (*Osmia bicornis*) was unaffected after exposure to 10 mg/g of glyphosate (Strobl et al. 2020). Production of royal jelly, that is consumed by hive larva, was reduced after weekly exposure to 6.68 mg/L of glyphosate for one month (Chaves et al. 2020). Vazquez et al. (2020b) showed that glyphosate exposure (2.5 mg/L) increased the proportion of delayed or altered development by 18% in bee larva, but this had no effect on survival. In a study on colony health and survival, Odemer et al. (2020) observed few serious effects; decreases in brood development rate and hatching weight at experimental high-dose exposure (137 mg/kg for 21 days) were observed. However, similar exposure in the field had no effects.

Studies on the bee gut microbiome were conducted by Motta et al. (2020) and Motta & Moran (2020). When young bees were chronically exposed to a range of glyphosate concentrations, there were few effects on overall bacterial abundance, but abundance of a core member of the bee gut microbiome (*Snodgrassella alvi*) was reduced. Decreased survival was observed at concentrations above 16.91 mg/L, but this was unlikely a result of perturbations in the gut microbiome (Motta & Moran 2020). Another study on the microbiome, conducted by Motta et al. (2020), also found that *Snodgrassella alvi* abundance was negatively affected by both oral and topical exposure to glyphosate. As with other animals, the bee gut microbiome has been linked to various aspects of health and bodily function. Although glyphosate has been shown to alter the composition of bee guts, it is unclear if and how these alterations are impacting overall health. Lab and field data on hive return rates, pathogen infection, and mortality were also investigated by Motta et al. (2020). Following exposure to glyphosate, the number of bees that returned to the hive decreased by about 16% when orally exposed and by as much as 41% when exposed topically. Much like the findings of Faita et al. (2020), bees that had been exposed to glyphosate then infected with *Serratia marcescens* (a harmful bacteria) had higher mortality rates than those that were not exposed to glyphosate. Mortality, as a result of topical exposure to 0.5% Roundup and above, increased within 24 hours (Motta et al. 2020). Pathogens, such as the ones studied by Faita et al. (2020) and Motta et al. (2020), can have serious effects on colony health. Both studies showed that glyphosate exposure can exacerbate the effects of pathogens and compromise a hive's ability to withstand infection.

Impacts on bee activity and sleep were reported in two papers (Delkash-Roudsari et al. 2020 & Vazquez et al. 2020a). It is thought that reduced duration and quality of sleep can impair learning in bees. Vazquez et al. (2020a) found that at 50 ng of glyphosate, bees may have slept more deeply. Delkash-Roudsari et al. (2020) showed that daytime and nighttime activity were reduced by 0.12-24 mg/L of glyphosate, but learning was unaffected. Almasri et al. (2020) found modulated activity for the enzymes CaE-3, GST, AChE, G6PDH, and ALP in bees that survived chronic glyphosate exposure.

There is minimal evidence regarding the levels of glyphosate that bees may be exposed to in the wild. Nectar and pollen are a likely source of glyphosate exposure, as well as residues in honey. In a greenhouse experiment, Thompson et al. (2014) found that glyphosate residues in nectar were between 2.78-31.3 mg/kg and 87.2-629 mg/kg in pollen after spraying flowering plants directly with 2.88 kg/ha of glyphosate; residues declined over time. This is one of the few studies to evaluate glyphosate residues in pollen and nectar, but it represents a worst-case exposure scenario. Plants were sprayed directly with glyphosate and bees were restricted to foraging on treated plants immediately after exposure. The studies presented above do not provide conclusive evidence that glyphosate exposure reduces survival or reproduction in bees. However, to minimize exposure to glyphosate-treated plants, MROSD should consider applying herbicides to flowering plants before or after peak bloom or during times of the day when bees are less active.

2.1.2.5 Terrestrial Invertebrates

Invertebrates are essential to the function of terrestrial ecosystems. They are present throughout the landscape and serve as pollinators, decomposers, herbivores, and food sources for other organisms. Due to their diverse ecosystem functions, invertebrates can come into contact with herbicides via aerial application, runoff, and residues on plants. Therefore, it's important to understand the extent to which glyphosate may impact invertebrate populations. In 2020, there were 15 studies evaluating the effects of glyphosate on the health, physiology, and function of terrestrial invertebrates. Common earthworms (*Lumbricus terrestris* and *Eisenia fetida*) were unaffected by glyphosate-based herbicides. Chronic exposure at a dose of 0.52 mg/kg did not alter body mass or cocoon number of *L. terrestris* (Nuutinen et al. 2020). Similarly, biomass and survival of *E. fetida* were not affected by Roundup, but they were affected by 26.3 mg/kg of pure glyphosate (Pochron et al. 2020). Owagboriaye et al. (2020) and Samal et al. (2020) showed that glyphosate altered the physiology of exotic earthworms at doses of 0.1 g/kg and 83.2 g/m^2, and springtails (*Sminthurinus niger*), another decomposer, were more active after exposure to pure and formulation glyphosate (Maderthaner et al. 2020). Nuutinen et al. (2020) and Maderthaner et al. (2020) also showed that glyphosate had no effects on the rate of invertebrate litter decomposition. At relevant concentrations, glyphosate exposure is of little concern to invertebrate decomposers. Glyphosate exposure had varied effects on flying insects. Development, feeding behavior, reproduction, and malaria (*Plasmodium relictum*) infection in mosquitoes (*Culex pipens*) were unaltered by 0.05 or 0.10 mg/L of glyphosate; in a separate study mosquito larval abundance decreased after exposure to 0.6 mL/m^2 of Roundup (plus adjuvant) (Bataillard et al. 2020 and Portilla & Lawler 2020). Roundup and a surfactant (polyethoxylated tallowamine), but not pure glyphosate, reduced survival and fecundity in fruit flies when applied at 15 µg/mL (Bednarova et al. 2020). A study on the potato beetle (*Leptinotarsa decemlineata*) revealed some effects of 6.4 L/ha of Roundup on antioxidant enzyme activity and gut microbial relative abundance, but not microbial diversity or richness (Gomez-Gallego et al. 2020). At the same concentration, Rainio et al. (2020) found no reduction in beetle survival or body mass. Ants (*Crematogaster scutellaris*) avoided food contaminated with 0.036-36 g glyphosate/L of Roundup in a dose dependent manner (Frizzi et al. 2020). Alhewairini (2020) found that five species of mites experienced a dose-dependent increase in mortality when directly sprayed with 0.5x, 1x, and 2x of the field dose of glyphosate-based herbicide.

Given that few of the studies published in 2020 reported adverse effects of glyphosate on invertebrate survival, glyphosate use poses minimal threat to terrestrial invertebrates, especially considering the potential negative impacts that invasive species may have on these organisms.

2.1.2.6 Amphibians

Nine papers were published in 2020 evaluating the impacts of glyphosate on amphibians. However, none of the studies included taxa that occur within MROSD jurisdiction. Areas of study included mortality, growth, reproduction, enzymatic activity, and behavior. Of the nine papers, two considered the effects of relevant concentrations of glyphosate on survival (Herek et al. 2020 & Agostini et al. 2020). Herek et al. (2020) was the only study to report that environmentally realistic concentrations led to increased mortality. However, mortality was never greater than 15%

despite 14 days of chronic exposure. They also found dose-dependent increases in external and internal malformations within this time frame. Although Herek et al. (2020) considered realistic concentrations of glyphosate, their study design was not representative of natural fluctuations/degradation of glyphosate in the environment. Moutinho et al. (2020) used amounts 8 times the relevant concentrations when chronically exposing tree frog (*Boana pardalis*) larva to glyphosate and still observed no more than 20% mortality. Conversely, a field study of four species of tadpoles inhabiting ponds contaminated with up to 315 µg/L of glyphosate showed no reductions in survival but did show a reduction in mobility after 48 hours (Agostini et al. 2020).

Though not immediately deadly to amphibians at relevant doses, glyphosate can cause a variety of sub-lethal effects that could potentially result in population level impacts. Analyses of amphibian response to glyphosate exposure produced a variety of results. Minimal liver damage, specifically relating to lipid vesicles, occurred in tree frog (*Dendropsophus molitor*) tadpoles following exposure to 325 and 750 µg glyphosate/L of Roundup (Riano et al. 2020). However, in the wild it is not likely that amphibians would encounter glyphosate at these levels. Bolis et al. (2020) found that growth and duration of tadpole activity, but not antipredator response, of marsh frogs (*Pelophylax ridibundus*) was sometimes affected by exposure to 7.6 mg/L glyphosate. Conversely, chronic exposure to 2.4 mg/L of glyphosate had no effects on the activity rates, growth, or development of tree frog (*Boana pardalis*) larva (Moutinho et al. 2020). At high concentrations, Roundup stunted embryonic growth and increased abnormalities in unfertilized eggs of African tree frogs (*Xenopus laevis*) (Turhan et al. 2020 & Slaby et al. 2020). In an analysis of pesticide avoidance behavior, Leeb et al. (2020) found that European toads (*Bufo bufo*) were more mobile and spent significantly less time on surfaces contaminated with 10% and 100% of the maximum field application rates of a glyphosate-based herbicide. This suggests that amphibians may intentionally avoid areas containing pesticide residues.

Taken together these studies suggest that lethality caused by glyphosate exposure is of little concern in environmentally realistic scenarios. However, sub-lethal impacts, such as reductions in mobility and structural abnormalities have been documented, though not definitively. In general, no serious effects were reported. The variable outcomes of the studies presented here suggest the need for field-based studies that expose amphibians to concentrations that they would encounter following applications in natural or agricultural environments. More studies are needed that evaluate how non-target effects of glyphosate on amphibian physiology and behavior result in changes in survival or reproduction.

2.1.2.7 Fish

The majority of studies published on fish from 2020 assessed how glyphosate alters physiology, specifically the activity of enzymes, hormones, and proteins. These compounds are useful biomarkers for determining how organisms react to foreign compounds such as glyphosate, especially when physical indicators are absent. For example, Bonifacio et al. (2020) found no effects of 0.2 or 2 mg/L of Roundup on health or mobility in the ten spotted live-bearer (*Cnesterodon decemmaculatus*), but cortisol levels were reduced by the lower dose. Cortisol is a steroid that is secreted in response to stress; when inhibited it may impact an organism's ability to respond to stressors. Embryonic exposure to 1, 2, or 5 µg glyphosate/mL of Roundup had no effect on cortisol levels, social or exploratory behavior, hatching rate, or survival of zebrafish (*Danio rerio*) larva (Lanzarin et al. 2020). For slender rasbora (*Rasbora daniconius*), chronic exposure to 0.56 mg/kg of Roundup increased three metabolic enzymes: ALP, AST, and ALT (Kharat et al. 2020). These enzymes are commonly used as stress biomarkers, and their increased activity is indicative of liver damage. Aminov & Golovanova (2020) found that in vitro exposure to 25 µg glyphosate/L of Roundup reduced the activity of the digestive enzyme amylase in 5 species of fish, though effects were temperature- and pH-dependent. Furthermore, when common carp (*Cyprinus carpio*) and Caspian kutum (*Rutilus kutum*) were exposed to 10% and 20% of their 96 hr LC_{50} concentration of glyphosate (520.77 mg/L and 6.64 mg/L, respectively) there were negative physiological effects; cytokines (immune response biomarkers) were altered in carp and AChE activity (a neurological biomarker) was altered in kutum fries (Ma & Li 2020 and Shiry et al. 2020). Membrane protein activity increased in the gills, gut, and liver when zebrafish were exposed to 0.1 mg/L of glyphosate (Moraes et al. 2020). There was only one study examining effects on morphology and they observed minimal impacts on gill structure (Menendez-Helman et al. 2020).

In an analysis of anti-predator behavior in zebrafish, both Lanzarin et al. (2020) and Pompermaier et al. (2020) found evidence that exposure to glyphosate compromises the response to predatory cues. This is of some concern as it suggests that fish exposed to glyphosate could be more vulnerable to predation.

Though no studies reported direct effects of glyphosate on survival, sub-lethal impacts are apparent. The numerous and varied effects on physiological components are difficult to draw conclusions from. Enzymes, hormones, and proteins are crucial to bodily function, but it is unclear if glyphosate-induced alterations in these compounds result in significant or permanent damage.

2.1.2.8 Aquatic Invertebrates

In 2020, nine papers were published outlining the impacts of glyphosate on marine and freshwater invertebrates. When sea urchin embryos were exposed to 1, 10, 50, and 100 µg/L of pure glyphosate, AMPA (aminomethylphosphonic acid; a glyphosate degradation product), or Roundup, delays in larval development, increased frequency of malformations, and increased rates of respiration occurred (Asnicar et al. 2020). Freshwater copepods (*Notodiaptomus carteri*) were also developmentally delayed as a result of glyphosate exposure. However, in this case organisms were exposed for 30 days at a minimum concentration of 0.38 mg/L (Fanton et al. 2020). At exceedingly high concentrations, Suppa et al. (2020) reported genotoxicity, reduced growth, maturation, development, and fecundity, and alterations in the gut microbiome of *Daphnia magna*. Multiple studies investigated how enzymes and proteins respond to glyphosate. Enzymes and hormones involved in the molting process of the freshwater prawn (*Macrobrachium potiuna*) were reduced after 14 days of exposure to 0.0065, 0.065, and 0.28 mg glyphosate/L of Roundup (de Melo et al. 2020b). A companion study found that antioxidant protein expression was also altered, but effects were sex-dependent; expression was up-regulated in females and down-regulated in males (de Melo et al. 2020a). Fabrello et al. (2020) showed similar sex-dependent effects of glyphosate exposure in mussels. Vitellogenin, a protein involved in the reproductive cycle, was reduced in female gonads and increased in male gonads as a result of chronic glyphosate exposure. Immune activity was altered in both crayfish (*Pontastacus* *leptodactylus*) and European abalone (*Haliotis tuberculata*) at high glyphosate concentrations (Banaee et al. 2020 & Mottier et al. 2020). A large-scale field study reported that 85 µg of glyphosate stream inputs had no effect on macroinvertebrate abundance, richness, or diversity (Xiang et al. 2020).

The studies reported here provide evidence on the sub-lethal effects of non-target glyphosate exposure. At varying concentrations, glyphosate exposure resulted in developmental delays or complications. Given these results, it is important that glyphosate be used minimally and cautiously when in proximity to waterways.

2.1.2.9 Aquatic Microbes

Microbes, such as plankton and bacteria, are foundational members of aquatic food webs. Their response to pollutants and nutrient inputs can affect organisms throughout the ecosystem. Seven papers were published in 2020 analyzing the effects of glyphosate on aquatic microorganisms. For adult macroalga (*Carpodesmia crinita*), long-term exposure to 1 and 10 µg/L of glyphosate had no effect on health or fecundity, but recruitment was substantially reduced (de Caralt et al. 2020). Recruitment dynamics have important implications for population stability; if these effects were to persist, *C. crinita* populations would suffer. de Carvalho et al. (2020) found that growth and metabolism of a marine diatom were affected by glyphosate concentrations of 250 µg/L and above over a 96-hour exposure period. Due to the short observational period, it is unclear whether or not diatoms are permanently affected by glyphosate exposure. In a comparison of uncontaminated and glyphosate-polluted lakes, Castro Berman et al. (2020) showed that picocyanobacteria abundance was greater in contaminated versus uncontaminated lakes. However, glyphosate concentration was not a reliable predictor of abundance. The response of autotrophic picoplankton communities exposed to 4 mg/L of glyphosate for 8 days was influenced by turbidity. In high turbidity, abundance decreased and community structure was unchanged, but in low turbidity abundance was unaffected and community structure was altered (Alejandra Sabio y Garcia et al. 2020). Silva et al. (2020) showed that cladocerans reared in warm temperatures are less sensitive to glyphosate exposure regardless of the temperature during exposure. In these cases, the influence of environmental variables on glyphosate impacts is apparent. Three studies in particular demonstrate the resiliency of microbial communities to glyphosate exposure. Lozano et al. (2020) found no effects of glyphosate on the abundance and productivity of a microbial community at concentrations of 0.3 mg/L. Even at high concentrations (3 mg/L) when effects were apparent, abundance and productivity stabilized within 23 days post-exposure. In another study, glyphosate exposure at concentrations of 2.5 mg/L led to changes in expression of genes related to metabolism, cellular processes, and informational processing, but expression was altered in a way that resulted in a net-zero effect on function; community composition was also unaffected (Lu et al. 2020). Fugere et al. (2020) showed that phytoplankton communities with previous exposure to glyphosate are capable of withstanding additional high-intensity exposures. Communities conditioned by glyphosate were able to maintain their biomass even after exposure to doses of 80 mg/L and above. No studies published in 2020 provide compelling evidence to suggest that glyphosate has persistent negative effects on aquatic microbial communities.

2.1.2.10 Soil Microbes

In general, glyphosate had little to no effects on soil microbial community structure or dynamics. Three studies from 2020 reported no effects of glyphosate on microbial communities at a range of concentrations, including 49 µg/g, 10 mL/L, and 0.87 kg/ha (Allegrini et al. 2020, Bottrill et al. 2020, and Kepler et al. 2020). The remaining studies demonstrated that glyphosate can perturb the soil microbiome, but often times other environmental variables are equally, if not more influential than glyphosate. Tree size and time of year influenced the effects of glyphosate on the abundance and richness of fungal communities associated with black cherry (*Prunus serotina*; Korzeniewicz et al. 2020). Gornish et al. (2020) showed that soil microbial communities were driven primarily by the presence of an invasive plant (*Cenchrus ciliaris*), rather than glyphosate. The soil bacterium *Bacillus* sp. experienced a reduction in colony growth after exposure to 7.2 mg/mL of glyphosate, but its inhibitory interaction with the pathogenic fungus *Fusarium* sp. was unaffected (Goncalves et al. 2020). Wilkes et al. (2020) found that tilling and glyphosate application inhibited the biomass and root symbiosis of arbuscular mycorrhizal fungi in wheat plants.

2.1.2.11 Plants

Wild plants are perhaps are the most susceptible to non-target glyphosate exposure. As a broad-spectrum herbicide, glyphosate similarly effects native and non-native plant species. When native non-target plants were exposed to a small fraction of the recommended field dose for glyphosate-based herbicides, they experienced reductions in leaf length, flowering height, number of flowers, and pollinator visitation (Carpenter et al. 2020 & Russo et al. 2020). Herbicide spillover is common in agricultural settings, particularly when crop lands and wild areas are in close proximity, and when indiscriminate application methods are used. Selective application methods used by MROSD reduce the effects of glyphosate on-target effects plants. However, the use of broad spectrum herbicides in areas with high abundance of intermingled native plants is not recommended.

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. 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. Herbicide usage is generally more effective than other methods of plant control (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 minimal, using glyphosate over other herbicides is the safest option to date.

Glyphosate is heavily utilized in agriculture, and its use in farming differs significantly from that in natural areas. Seasonal application and aerial sprayers, as well as other non-selective machinery used in agriculture, increase the likelihood of non-target exposure and persistence of glyphosate in the environment. Additionally, glyphosate resistant crops enable frequent and intense usage of glyphosate-based herbicides. The precise herbicide application techniques practiced by MROSD vary drastically from agricultural practices. Direct application to target plants and consideration of rainfall events prior to application, greatly reduce the chances of spillover thereby avoiding impacts on non-target organisms.

A majority of the studies published in 2020 evaluated glyphosate exposure at concentrations that exceeded even the highest levels of glyphosate that have been recorded in the environment. The findings summarized in this report indicate that sublethal effects can occur as a result of exposure. Non-lethal effects on various parameters relating to activity, growth, and physiology were documented. However, few studies were able to illustrate how exposure would result in population level impacts. In many cases, organisms exhibited biochemical responses (enzyme, hormonal, genetic, etc.) to glyphosate exposure, but whether or not these responses are specific to glyphosate or merely generic reactions to environmental stress is unclear. Additionally, the impact of glyphosate on survival and reproduction is of crucial importance for determining its impact on non-target organisms, and many studies are missing this analysis. There is also a lack of data regarding recovery or long-term effects of sublethal exposure. Based on the findings published in 2020 and included in this report, we see no conclusive evidence that the judicious use of glyphosate in natural areas poses a serious threat to the viability of non-target organisms.

2.2 *IMAZAPYR*

No papers were published in 2020 describing the human health effects, ecological risk, or environmental fate of imazapyr. Three studies were published in 2020 that described the efficacy of imazapyr use to control invasive plants (Barksdale et al. 2020, Prince & Macdonald 2020, and Vogt et al. 2020) and are outlined in the annotated bibliography.

2.3 *AMINOPYRALID*

No papers were published in 2020 describing the human health effects, ecological risk, or environmental fate of aminopyralid. Three studies were published in 2020 that described the efficacy of aminopyralid use to control invasive plants (Aulakh 2020, Bobadilla et al. 2020, and Vogt et al. 2020) and are outlined in the annotated bibliography.

2.4 *TRICLOPYR*

No papers were published in 2020 describing the environmental fate of triclopyr. One study evaluated the non-target effects of triclopyr on oil palm pollinators. Setyawan et al. (2020) investigated how pesticide residue in oil palm cultivation affected a pollinating weevil. Glyphosate (480g/L) and triclopyr (670g/L) were both applied at 0.3% and resulted in 33% and 63% mortality, respectively. Two studies were published in 2020 that described the efficacy of triclopyr use to control invasive plants (Patton et al. 2020 and Vogt et al. 2020) and is outlined in the annotated bibliography

2.4.1 Human Health

In 2020, one paper investigated the relationship between pesticide exposure and the risk of prostate cancer. Pardo et al. (2020) used the American Health Study, which includes self-reported pesticide use from 1993-2010 to evaluate the incidence of prostate cancer among pesticide applicators. They found that pesticide applicators who used triclopyr actually had a lower risk of developing prostate cancer. They also considered clethodim and clopyralid and found no link between exposure to these pesticides and prostate cancer. Their analysis evaluated pesticide use as never or ever using the pesticide and therefore was unable to account for the frequency of use, severity of exposure, or use of PPE.

2.5 *CLOPRYALID*

Apart from Pardo et al. (2020) (see Section 2.4.1), there were no papers published in 2020 that described the human health effects, ecological risk, or environmental fate of clopyralid. One study was published in 2020 that described the efficacy of clopyralid use to control invasive plants (Aulakh 2020) and is outlined in the annotated bibliography.

2.6 *CLETHODIM*

Other than Pardo et al. (2020) (see Section 2.4.1), no papers published in 2020 described the human health effects or environmental fate of clethodim. Two studies assessed the non-target impacts of clethodim on toxin-producing cyanobacteria (*Microcystis aeruginosa*) and non-target plants (Breda-Alves et al. 2020 and Siddiqui & Al-Rumman 2020). When exposed to high doses of clethodim (≥50 mg/L) for 4 days cell density decreased, and toxin content increased for cyanobacteria (Breda-Alves et al. 2020). Siddiqui & Al-Rumman (2020) found that when pea (*Pisum sativum*) seeds were exposed to 0.01-0.04% of clethodim, mature plants had an increased incidence of pollen sterility that was as high as 83%. Five studies were published in 2020 that evaluated the efficacy of clethodim use to control invasive plants (Barksdale et al. 2020, Bianchi et al. 2020, Bowen et al. 2020, Brunori & Puricelli 2020, and Metier et al. 2020) and are described in the annotated bibliography.

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

No papers were published in 2020 describing the human health effects, ecological risk, or environmental fate of the above listed substances when used as adjuvants or surfactants.

4.0 FUNGICIDE

4.1 *PHOSPHITE K*

No papers were published in 2020 describing the human health effects, ecological risk, or environmental fate of phosphite K.

5.0 INSECTICIDES

5.1 *DIATOMACEOUS EARTH*

No papers were published in 2020 describing the human health effects, ecological risk, or environmental fate of diatomaceous earth when used as an insecticide.

5.2 *D-TRANS ALLETHRIN*

No papers were published in 2020 describing the human health effects, ecological risk, or environmental fate of D-trans allethrin when 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. Fipronil is the active ingredient in Maxforce bait stations used by MROSD to control Argentine ants. Fipronil bait stations are secure pesticide reservoirs used where termites, roaches, or ants occur in or around structures. MROSD places bait stations exclusively indoors, thereby posing no consequential threat to non-target plants or animals. 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. The studies summarized in this section evaluate risk associated with bait station applications of fipronil.

5.3.1 Human Health

In 2020, one paper investigated the human exposure risk associated with fipronil. Beranger et al. (2020) quantified pesticide exposure in small cohort of French mothers by determining the pesticides present in hair samples. They also recorded the size of their children at the time of birth. They found that mothers whose hair contained the fipronil metabolite, fipronil sulfone, had children with greater than average birth length. Due to the relatively small sample size and the lack of data showing direct effects of fipronil on birth measurements, it would be misleading to suggest that fipronil influences birth size. Additionally, the methods of exposure are not outlined and cultural differences in pesticide use make it difficult to apply this study to US applicators.

5.3.2 Non-target

Bae & Kwon (2020) assessed the effects of fipronil on the function of mouse sperm. Sperm exposed to 1 µM of fipronil had reduced motility, and when exposed to 300 µM fertilization ability was negatively affected. However, this is not directly relevant to MROSD because they do not address non-target affects resulting from the use of fipronil bait stations.

5.3.3 Environmental Fate

In 2020, there were multiple (16) studies published evaluating the effects of fipronil on non-target organisms and its persistence in the environment. However, all these studies consider fipronil use in agricultural, veterinary, or residential applications. None of them address the non-target or environmental effects of fipronil when used as indoor bait traps and are therefore not described here.

5.4 *INDOXACARB*

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

No papers were published in 2020 describing the human health effects of indoxacarb. Two published studies evaluated the effects of indoxacarb on non-target animals and one study assessed the dissipation and persistence of indoxacarb in the environment. However, all of these studies considered extensive indoxacarb use in agricultural and forestry applications. They do not describe how indoxacarb may enter the environment or come in contact with non-target organisms when used in indoor bait traps and therefore are not described here.

5.5 *PHENOTHRIN*

No papers were published in 2020 describing the human health effects, ecological risk, or environmental fate of phenothrin.

5.6 *PRALLETHRIN*

No papers were published in 2020 describing the human health effects, ecological risk, or environmental fate of prallethrin.

5.7 *S-HYDROPRENE*

No papers were published in 2020 describing the human health effects, ecological risk, or environmental fate of S-hydroprene.

5.8 *SODIUM TETRABORATE DECAHYDRATE*

No papers were published in 2020 describing the human health effects, ecological risk, or environmental fate of sodium tetraborate decahydrate.

6.0 RODENTICIDES

6.1 *CHOLECALCIFEROL*

No papers were published in 2020 describing the human health effects, ecological risk, or environmental fate of cholecalciferol when used as a rodenticide.

Organism	Findings Published in 2020	Reference
Mammals		
Cow	Embryos exposed to ≥0.9 ppm of Roundup experienced delayed development, increased cellular stress, and cell death.	Cai et al. 2020
	Roundup exposure did not affect blood cells, enzyme activity, or genotoxicity.	Schnabel et al. 2020
Pig	228 g glyphosate/day of Roundup had no effect on gut microbiota.	Krause et al. 2020
	Motility, viability, and cellular function of pig sperm was altered by both pure glyphosate and Roundup, although Roundup was found to be more toxic. Glyphosate was only effective at 360 µg/mL, but impacts of Roundup occurred at concentrations as low as 5 g/L	Nerozzi et al. 2020
	Piglets that consumed Roundup contaminated food at concentrations of 20 mg/kg and above experienced increased activity of antioxidant enzymes (CAT, SOD, and MDA) and altered gene expression in the intestine. Morphology was not affected.	Qiu et al. 2020
	Roundup, but not glyphosate, reduced progesterone levels and increased oxidative stress in pig ovarian cells at concentrations exceeding 100 µg/mL. Roundup and glyphosate reduced blastocyst development.	Spinaci et al. 2020
Ewe lamb	Lambs injected with 2 mg glyphosate/kg of Roundup had altered cell function and gene expression in uterine tissues, but uterine morphology was not affected.	Alarcon et al. 2020
Birds		
Chicken	Intestinal morphology and antioxidant enzyme activity of the liver and small intestine altered by injection of 10 mg glyphosate/kg of Roundup or glyphosate into chicken eggs. Total embryo weight was not affected.	Fathi et al. 2020
Japanese quail (*Coturnix japonica*)	Exposure to Roundup contaminated feed at a dose of 160 mg/kg for 52 weeks increased richness and abundance of gut microbiota and altered liver catalase activity. There were no effects on body mass or reproduction.	Ruuskanen et al. 2020
Japanese quail (*Coturnix japonica*)	No evidence that parental exposure to glyphosate had effects on offspring health.	Ruuskanen et al. 2020a
Reptiles		
Tegu lizard (*Salvator merianae*)	Antibodies and white blood cell type of embryos were altered by glyphosate-based herbicides at concentrations at or exceeding 400 µg/egg. White blood cell count was not affected.	Mestre et al. 2020
Bees		

Organism	Findings Published in 2020	Reference
Honey bee (*Apis mellifera*)	When chronically exposed to 0.1, 1, and 10 µg/L of glyphosate, bees had lower survival rates and increased food consumption. Enzyme activity was modulated throughout the experiment, but more so at day 10 than day 20.	Almasri et al. 2020
	Royal jelly production was reduced by 47% when hives were given 6.68 mg glyphosate/L of Roundup once a week for one month.	Chaves et al. 2020
	Exposure to glyphosate (0.12 - 0.24 mg/L) had varying, but minimal effects on bee activity, specifically circadian rhythm, but did not affect survival.	Delkash-Roudsari et al. 2020
	Bees were exposed to a diet of Roundup and/or *Nosema sp*. (a harmful microsporidium) for 120 hours. Individually Roundup, at a dose of 2.16 µg glyphosate/g feed, and *Nosema* had no significant effect on survival or food consumption. However, when applied together, they collectively increased food consumption and reduced survival by at least 20%.	Faita et al. 2020
	When bees were orally or topically exposed to 0.1% - 3% Roundup, in the lab and in the field, hive recovery rates decreased, gut microbial abundance was reduced, and mortality increased. They also detected glyphosate in the honey of treated hives and found that glyphosate exposure can affect the severity of *Serratia* infection.	Motta et al. 2020
	Core members of the gut microbiome, specifically *Snodgrassella alvi*, had reduced abundance after exposure to 1.691 mg/L - 169.1 mg/L of glyphosate. Bee survival decreased at doses above 16.91 mg/L.	Motta & Moran 2020
	Colony health was minimally affected by chronic high-dose (137 mg glyphosate/kg) exposure to a glyphosate-based herbicide in experimental conditions. No effects were observed in the field.	Odemer et al. 2020
	Exposure to 0-100 ng of glyphosate had minimal effects on bee sleep.	Vazquez et al. 2020a
	Glyphosate increased the incidence of developmental delays in larva exposed at a dose of 2.5 mg/L. In larva with no physical symptoms, some changes in the expression of metabolic and environmental stress biomarkers were observed.	Vazquez et al. 2020b
Osmia bicornis	Survival and feeding behavior of adult bees was unaffected by exposure to 1.0 x 10^7 ng/g of glyphosate and/or 1.5 ng/g of clothianidin.	Strobl et al. 2020

Organism	Findings Published in 2020	Reference
Terrestrial Invertebrates		
Mites *(Lasioseius dentatus)* *(Androlaelaps casalis)* *(Rhodacarus roseus)* *(Macrocheles muscaedomesticae)* *(Cunaxa setirostris)*	Mortality increased in a dose-dependent manner when mites were sprayed directly with 0.5x, 1x, and 2x of the recommended field dose of a glyphosate-based herbicide (1.1L/100L).	Alhewairini (2020)
Solenopsis sp. *Hypogastrura* sp. *Bemisia tabaci* *Caliothrips brasiliensis* *Cerotoma arcuatus* *Tetranychus* sp	Application of 1,080 g/ha of glyphosate decreased the densities of *Solenopsis* sp. and *Hypogastrura* sp. and increased the density of *Bemisia tabaci, Caliothrips brasiliensis, Cerotoma arcuatus, Tetranychus* sp.	Pereira et al. 2020
Mosquito (*Culex pipens*)	Exposure to 0.05 and 0.10 mg glyphosate/L had no effects on larval development time, adult mosquito size, adult feeding behavior, number of eggs laid, or severity of malaria (*Plasmodium relictum*) infection.	Bataillard et al. 2020
	Mosquito larval abundance was reduced after exposure to 0.6 mL glyphosate/m^2 of Roundup + adjuvant.	Portilla & Lawler 2020
Fruit fly (*Drosophila melanogaster*)	Roundup (15 µg/L) and polyethoxylated tallow amine (45 µg/L) reduced survival, impaired negative geotaxis, reduced fecundity and cell viability, and increased carbonylated protein levels. Pure glyphosate (100µg/mL) had no effect.	Bednarova et al. 2020
Ant (*Crematogaster scutellaris*)	Ants avoided food contaminated with 0.036, 0.36, 3.6, 36 g glyphosate/L Roundup. The feeding frequency was dose-dependent, but never dropped below 60% except at the highest concentration.	Frizzi et al. 2020
Potato beetle (*Leptinotarsa decemlineata*)	Indirect exposure to 6.4 L/ha of Roundup affected relative abundance of gut microbiota, but not microbial diversity or richness.	Gomez-Gallego et al. 2020
	Oxidative status biomarkers (tGSH, CAT, SOD) were modulated by indirect exposure to 6.4 L/ha of glyphosate. Survival and body mass were not affected.	Rainio et al. 2020
Springtail (*Sminthurinus niger*)	Springtail activity increased when exposed to 2.18-2.43 kg/ha of glyphosate and was influenced by soil organic matter.	Maderthaner et al. 2020
Earthworm (*Lumbricus terrestris*)	61-day exposure to 0.52 mg/kg of a glyphosate-based herbicide had no effect on worm mass, number of cocoons, or incorporation of plant matter into the soil.	Nuutinen et al. 2020
Earthworm (*Alma millsoni*) (*Eudrilus eugeniae*) (*Libyodrilus violaceus*)	Enzyme (LDH, MT, GST) activity and oxidative biomarkers (CAT, SOD, GPX, GSH) were altered by 83.2 g glyphosate/m^2.	Owagboriaye et al. 2020

Organism	Findings Published in 2020	Reference
Earthworm (*Eisenia fetida*)	26.3 mg/kg of glyphosate, but not Roundup, reduced worm biomass and stress-test survival time, but did not affect microbial and fungal biomass.	Pochron et al. 2020
Tropical earthworm (*Drawida willsi*) (*Lampito mauritii*)	Exposure to glyphosate (0.1-0.2 g/kg) resulted in alterations in tissue protein levels, lipid peroxidation, and enzyme (LDH, AChE, and CAT) activity.	Samal et al. 2020
Soil Microorganisms		
	Microbial response to soil dry-rewetting effects was not impacted by 49 µg/g of herbicide application. Exposure caused a slight increase in respiration.	Allegrini et al. 2020
	Soil chemistry and microbial community structure were not altered by mulching or application of four herbicides, one of which was Roundup (10 mL/L).	Bottrill et al. 2020
	7.2 mg/mL of glyphosate reduced *Bacillus* colony growth but did not affect the interaction between *Bacillus* and *Fusarium*.	Goncalves et al. 2020
	Bacterial and fungal communities were primarily influenced by buffelgrass invasion. Glyphosate-based herbicides had minimal effects on the soil microbiome when mixed and applied at 3-5%.	Gornish et al. 2020
	Two applications of 0.87 kg/ha of glyphosate did not affect microbial community structure, *Fusarium* abundance, or crop yield.	Kepler et al. 2020
	The effects of 6 L/ha of glyphosate on *Prunus serotina* fungal communities was dependent the time of year and tree size.	Korzeniewicz et al. 2020
	When *Arabidopsis* seedlings were inoculated with microbes and treated with 3.6 µg/L of glyphosate for 12 days, root and shoot growth was reduced. The same dose without microbes present caused a growth increase.	Ramirez-Villacis et al. 2020
	Tillage and glyphosate exposure inhibited AM fungal biomass and root symbiosis.	Wilkes et al. 2020
Fish		
Common perch Burbot Pike Zander Wels catfish	Collected the intestinal mucosa and chyme from five species of fish and exposed it to 25 µg glyphosate/L of Roundup at various temperatures and pH. Activity of the digestive enzyme amylase was inhibited for all species, but severity of inhibition was pH- and temperature-dependent.	Aminov & Golovanova 2020
Cnesterodon decemmaculatus	Roundup had no effects on fish health or mobility at concentrations of 0.2 and 2 mg/L. A reduction in cortisol levels was observed at 0.2 mg/L but not at 2 mg/L. LDH, CPK, and AChE levels were unaffected.	Bonifacio et al. 2020
Rasbora daniconius	Chronic exposure to 0.56 ppm of Roundup negatively altered liver injury biomarkers, specifically the metabolic enzymes ALP, AST, and ALT.	Kharat et al. 2020

Organism	Findings Published in 2020	Reference
Zebrafish (*Danio rerio*)	Embryonic exposure to 1, 2, or 5 µg glyphosate/mL of Roundup had no effect on social or exploratory behavior, hatching rate, cortisol levels, or survival of zebrafish larva. Predator avoidance behavior decreased in group exposed to 5 µg/mL.	Lanzarin et al. 2020
	Roundup and pure glyphosate had similar effects on the activity of membrane proteins after 96 hrs of exposure to 0.1 mg glyphosate/L. Both increased protein activity in the gills, gut, and liver.	Moraes et al. 2020
	Exposure to the LC50 concentration of a glyphosate-based herbicide (5.2 mg/L) at a rate of 10%compromised responses to predatory stimulus. Fish entered the central, open area more frequently and spent more time there, increasing their vulnerability to predation.	Pompermaier et al. 2020
Common carp (*Cyprinus carpio*)	Fish were exposed to 10% and 20% of the 96hr LC50 concentration of glyphosate (520.77 mg/L) for up to 168 hours. Proinflammatory cytokines (immune response biomarkers) were altered in the liver, kidneys, and spleen. Physical damage was reported but was not statistically analyzed.	Ma & Li 2020
Odontesthes bonariensis	Gill specific surface area increased when fish were chronically exposed to 1 mg glyphosate/L, but not 10 mg glyphosate/L, of a glyphosate-based herbicide. Other physical effects were not accompanied by statistical analysis.	Menendez-Helman et al. 2020
Rutilus kutum	Acetylcholinesterase activity was altered by chronic exposure to 10% and 20% of the 96hr LC50 concentration of glyphosate (6.64 mg glyphosate/L) in *R. kutum* fries.	Shiry et al. 2020
Amphibians		
Frog (*Boana pulchella*) (*Leptodactylus latrans*) Toad (*Rhinella fernandezae*) (*Rhinella arenarum*)	Frogs/toads in ponds adjacent to cropland were monitored following pesticide application. Pesticides occurred both individually and in combination; concentrations of endosulfan, cypermethrin, glyphosate, and 2,4-Dichlorophenoxyacetic acid ranged from 0-365 µg/L. Tadpole mobility was reduced in all ponds and survival decreased in all ponds except those with only glyphosate.	Agostini et al. 2020
Frog (*Boana pardalis*)	Concentrations of 2.4 mg/L of glyphosate increased larval mortality but had minimal sublethal effects. Final mass, developmental stage, and activity rates were not affected, but there was an increase in AChE activity.	Moutinho et al. 2020
Toad (*Bufo bufo*)	Toads moved more frequently and spent less than 50% of their time on areas contaminated with 10% or 100% of the maximum recommended application rate of a glyphosate-based herbicide.	Leeb et al. 2020

Organism	Findings Published in 2020	Reference
Marsh frog (*Pelophylax ridibundus*)	Embryonic exposure to 7.6 mg glyphosate/L of Roundup reduced total tadpole length. Basal activity and rotational preference were affected in 1 of 2 behavior trials. Antipredator response was unaffected.	Bolis et al. 2020
Frog (*Physalaemus cuvieri*) (*Physalaemus gracilis*)	Chronic exposure to Roundup lead to dose-dependent increases in internal and external malformations (65-1000µg/L); length and mass of tadpoles decreased at Roundup concentrations ≥500 µg glyphosate/L. Although mortality did increase, it never exceeded 14% even at the highest concentrations.	Herek et al. 2020
Frog (*Dendropsophus molitor*)	Liver damage in tadpoles was assessed after chronic exposure to 325 and 750 µg glyphosate/L of Roundup. Only the number and area of lipid vesicles was affected.	Riano et al. 2020
Frog (*Xenopus laevis*)	Roundup was reported to have a 96 h LC50 of 32.1 mg glyphosate/L (embryos) and 35.1 mg glyphosate/L (stage 46 tadpoles) and stunted embryonic growth. Pure glyphosate had no effects at concentrations up to 500 mg/L.	Turhan et al. 2020
	High doses of Roundup (1480 µg glyphosate/L) induced oocyte maturation and increased the percentage of cellular abnormalities. There was no effect on maturation signaling pathways.	Slaby et al. 2020
Aquatic Invertebrates		
Sea urchin (*Paracentrus lividus*)	Embryonic exposure to of 1, 10, 50, and 100 µg/L of glyphosate, AMPA, or Roundup led to delayed larval development, increased physical abnormalities, shortened skeletal rod length, and an increased respiration rate in sea urchin larva.	Asnicar et al. 2020
Freshwater crayfish (*Pontastacus leptodactylus*)	21-day exposure to 0.4 and 0.8 mg/L of glyphosate altered enzyme and immune activity, glucose/protein/cholesterol/triglyceride levels, and oxidative biomarkers.	Banaee et al. 2020
Freshwater prawn (*Macrobrachium potiuna*)	Antioxidant protein expression was up-regulated in females and down-regulated in males after exposure to 0.0065, 0.065, and 0.28 mg glyphosate/L of Roundup for 7-14 days.	de Melo et al. 2020a
	Enzymes and hormones involved in molting were reduced when exposed to 0.0065, 0.065, and 0.28 mg glyphosate/L of Roundup for 14 days.	de Melo et al. 2020b
Mussel (*Mytilus galloprovincialis*)	A protein involved in the reproductive cycle was moderately affected by exposure to 100 and 1000 µg/L of glyphosate in Roundup after 21 days. The effects were greatest in male mussels.	Fabrello et al. 2020
Freshwater copepod (*Notodiaptomus carteri*)	30 days of 0.38 and 0.82 mg glyphosate/L disrupted development, but not body length or caloric content. Only females experienced increased GST and SOD activity.	Fanton et al. 2020

Organism	Findings Published in 2020	Reference
European abalone (*Haliotis tuberculata*)	Immune cells (hemocytes) negatively responded to glyphosate, Roundup, and an adjuvant. Immune viability, stability, and activity were reduced at concentrations above 1000 µg/L.	Mottier et al. 2020
Daphnia magna	Exposure to 1 mg/L of glyphosate and Roundup caused reduced growth, maturation, development, and fecundity. This dose was also found to be genotoxic and cause alterations in the gut microbiome.	Suppa et al. 2020
Freshwater macroinvertebrates	Roundup inputs had no effect on abundance, richness, or diversity of stream macroinvertebrates.	Xiang et al. 2020
Aquatic Microorganisms		
	Picoplankton (PP) communities were variably affected by 8 days of exposure to 4 mg/L of glyphosate. Heterotrophic PP community structure was altered. The response of autotrophic PP was dependent on turbidity. Abundance was affected only in high turbidity and community structure was affected only in low turbidity.	Alejandra Sabio y Garcia et al. 2020
	Abundance of picocyanobacteria (Pcy) differed between lakes with and without a history of glyphosate use, but the difference could not be explained by glyphosate concentration. Farming system and a phosphonate metabolizing gene were reliable predictors of Pcy abundance.	Castro Berman et al. 2020
Macroalga (*Carpodesmia crinita*)	Exposure to 1 and 10 µg/L of glyphosate for 6 months had no effect on adult health or fecundity. It did however reduce recruitment size and density.	de Caralt et al. 2020
Diatom (*Phaeodactylum tricornutum*)	Energy balance, protein and fatty acid content, cell density, and specific growth rate were affected by ≥250 µg/L of glyphosate.	de Carvalho et al. 2020
	Phytoplankton communities previously exposed to glyphosate responded positively to high-dose glyphosate exposure.	Fugere et al. 2020
	Low concentrations of a glyphosate-based herbicide (0.3 mg/L) had no effects on aquatic microbes. High concentrations (3 mg/L) caused perturbations in community structure and production, but the effects were short-lived.	Lozano et al. 2020
	Community composition of a freshwater microbiome was unaffected by 2.5 mg/L of glyphosate. Some transcriptional changes occurred, but the net effect was minimal.	Lu et al. 2020
Ceriodaphnia silvestrii	Animals reared in relatively warm water were more resilient to glyphosate-induced mortality than those raised in colder waters.	Silva et al. 2020

Organism	Findings Published in 2020	Reference
Plants		
	Flower number and flowering time of native plant species (*Centaurea cyanus, Silene noctiflora, Viola arvensis, Cerastium arvense, Cirsium arvense, Epilobium montanum, Knautia arvensis, Taraxacum officinale, Trifolium pratense*) were negatively affected when sprayed with 1 and 5% of the recommened field rate of five herbicides. Glyphosate had the greatest effects.	Carpenter et al. 2020
	Roundup had similar effects to organic herbicides when used to control invasive woody vines (*Akebia quinate, Euonymus fortune, Hedera helix, Vinca minor*). None of the herbicides had non-target effects on soil nematode abundance or total moss/fern spores.	Carreiro et al. 2020
	Mimicked a non-target exposure scenario for seven species of flowering plants (*Cirsium vulgare, Epilobium hirsutum, Plantago lanceolata, Origanum vulgare, Filipendula ulmaria, Hypochaeris radicata, Phacelia tanacetifolia*). When exposed to a glyphosate-based herbicide at 7% of the recommended field application rate, plants had reduced flowering height, leaf length, and pollinator visitation.	Russo et al. 2020

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APPENDIX A: ANNOTATED BIBLIOGRAPHY 2020

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1.0 HERBICIDES

1.1 *GLYPHOSATE*

HUMAN HEALTH

Agostini, L. P. *et al.* Effects of glyphosate exposure on human health: Insights from epidemiological and in vitro studies. *SCIENCE OF THE TOTAL ENVIRONMENT* 705, (2020).

In this literature review, Agostini et al. (2020) summarizes the known effects of glyphosate and glyphosate-based herbicides on human cell lines and human health. They describe the findings of 144 papers related to cells (both tumor and healthy), cancer, respiratory diseases, neurological effects, reproductive effects, and others. They show that many in vitro studies on human cell lines report at least some toxicity as a result of glyphosate exposure. The epidemiological effects were less conclusive. Though some health effects of glyphosate exposure were reported there was a lack of consensus and many studies failed to include information about glyphosate levels in the body, so there is uncertainty regarding the level of exposure. Overall, the authors conclude that more epidemiological studies, with standardized methodology, need to be conducted in order to accurately determine the human health effects of glyphosate exposure.

Donato, F., Pira, E., Ciocan, C. & Boffetta, P. Exposure to glyphosate and risk of non-Hodgkin lymphoma and multiple myeloma: an updated meta-analysis. *MEDICINA DEL LAVORO* 111, 63–73 (2020).

A meta-analysis performed by Donato et al. (2020) evaluated the relationship between glyphosate exposure and non-Hodgkin lymphoma (NHL) or multiple myeloma (MM). They used published studies describing the association between glyphosate exposure and cancer; seven studies of NHL totaling 1271 cases or deaths and three studies of MM totaling 290 cases or deaths were included. Glyphosate was treated as a binary variable (never vs. ever exposed), but dose response was evaluated when possible. They used risk ratios, where values greater than 1 with a confidence interval that does not overlap with 1 indicates a relationship between NHL or MM and glyphosate exposure. They report relative risks of 1.03 (95% CI 0.86-1.21) for NHL and 1.04 (95% CI 0.67-1.41) for MM, indicating that occupational exposure to glyphosate did not result in an increased risk of NHL or MM. When considering 3 studies that evaluated the extent of exposure, they found that the relative risk of NHL for the highest exposure category was 1.49 (95% CI 0.37-2.61). Additionally, they report a lack of negative results from small studies of NHL, indicating that publication bias may be influencing results.

Gillezeau, C., Lieberman-Cribbin, W. & Taioli, E. Update on human exposure to glyphosate, with a complete review of exposure in children. ***ENVIRONMENTAL HEALTH*** **19, (2020).**

In this update of Gillezeau et al. (2019), five new studies of urinary glyphosate levels are discussed. Gillezeau et al. (2019) found that exposure occurred at a wide range of concentrations in both the general population and in exposed workers, but major gaps in the literature have prevented researchers from determining the extent to which either group is generally exposed. Gillezeau et al. (2020) focused on populations that they deemed were most vulnerable: children and occupationally exposed adults. Urinary glyphosate levels ranged from 0.28 – 4.04 µg/L of glyphosate in children and adults. The authors conclude that studies on glyphosate exposure levels remain limited. Many studies lack information on severity of exposure, use of PPE, and urinary glyphosate levels prior to exposure. Additionally, data on urinary glyphosate levels in the general population is sparse, so it's difficult to say how those who are occupationally exposed differ from the general public.

Kougias, D. G. ***et al.*** **Risk Assessment of Glyphosate Exposures from Pilot Study with Simulated Heavy Residential Consumer Application of Roundup(R) using a Margin of Safety (MOS) Approach.** ***RISK ANALYSIS*** **doi:10.1111/risa.13646.**

Kougias et al. (2020) evaluated the internal doses corresponding to the urinary glyphosate levels measured by Peirce et al. (2020) following heavy application of glyphosate-based herbicides. They estimated internal glyphosate doses by measuring the amount of glyphosate residue found in urine. They report that all the estimated internal doses were much lower than even the most conservative values of acceptable daily intake. The daily dose did not exceed 6 µg/kg/day. **These results suggest that even in severe cases of glyphosate exposure, health risk is negligible.**

Mesnage, R. & Antoniou, M. N. Computational modelling provides insight into the effects of glyphosate on the shikimate pathway in the human gut microbiome. ***CURRENT RESEARCH IN TOXICOLOGY*** **1, 25–33 (2020).**

Mesnage & Antoniou (2020) studied the shikimate pathway in the human gut microbiome, and how it may be affected by glyphosate. The effectiveness of glyphosate is achieved by targeting and inhibiting the shikimate pathway, which ultimately leads to plant death, but this pathway is also present in microorganisms. Using genome and transcriptome profiles from the Human Microbiome Project (HMP), the authors measured the presence and activity of the shikimate pathway in gut microorganisms. Although they found genetic evidence suggesting that many microbes possess this biochemical pathway, there was little evidence indicating that this pathway is utilized. The purpose of the shikimate pathway in plants and microbes is to synthesize amino acids. The authors believe that gut microbes have access to these amino acids via host (human) food consumption and therefore do not need to synthesize it.

Mueangkhiao, P. *et al.* Biological variation in kidney injury and kidney function biomarkers among farmers in Lamphun province, Thailand. *ENVIRONMENTAL SCIENCE AND POLLUTION RESEARCH* 27, 12386–12394 (2020).

Mueangkhiao et al. (2020) measured kidney biomarker levels in Thai farmers that regularly apply herbicides. They interviewed 59 farmers regarding their pesticide use and lifestyle and collected blood and urine samples to measure levels of kidney function biomarkers. Farmers who had been diagnosed with kidney disease, diabetes mellitus, or hypertension were excluded from the study. Though all farmers reported using multiple pesticides at once, the most commonly used pesticide was glyphosate, and the majority of farmers reported using pesticides at least once a week. Pesticide exposure was calculated based on application method, PPE usage, pesticide mixing, and pesticide equipment repairs; these factors were used to generate an exposure intensity index (EII) score where low values indicate low exposure. They found that kidney biomarker levels were normal. However, they also found a positive association between glyphosate or EII and serum creatinine, a common kidney function biomarker. One important limitation of this study is that they did not quantify internal or urinary pesticide levels, so the levels and type of pesticide that was actually present in the body is unknown.

Pierce, J. S. *et al.* Pilot study evaluating inhalation and dermal glyphosate exposure resulting from simulated heavy residential consumer application of Roundup(R). *INHALATION TOXICOLOGY* 32, 354–367 (2020).

Pierce et al. (2020) quantified dermal and airborne glyphosate levels, and the resulting urinary glyphosate levels, for applicators in a high exposure scenario. Participants were placed in one of two groups: dermal (face respirator; no protective clothing) or inhalation (no face respirator; hooded Tyvek coveralls) exposure and applied Roundup with a backpack sprayer for 100min. Urine samples were collected from participants in both the dermal and inhalation exposure group 3, 6, 12, and 24 hours following glyphosate exposure. They found that **mean urinary glyphosate levels were highest 3 hours post-exposure and were significantly elevated following glyphosate application, but returned to baseline levels after 24 hours.** Some notable limits of this study include small sample size (n=12) and lack of replication.

Pu, Y. *et al.* Maternal glyphosate exposure causes autism-like behaviors in offspring through increased expression of soluble epoxide hydrolase. *PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA* 117, 11753–11759 (2020).

Pu et al. (2020) studied behavior and brain biochemical processes to evaluate the risk of Autism Spectrum Disorder (ASD) following maternal glyphosate exposure. Pregnant mice were administered 0.039 – 0.293% glyphosate during pregnancy and lactation. Researchers observed behavior of juvenile mice and measured select fatty acids, amino acids, and proteins that have been shown to be involved in the development of behaviors associated with ASD. When pregnant mice were exposed to 0.098% glyphosate, offspring showed "social interaction deficits". They also found that soluble epoxide hydrolase (sEH), which is suspected to play a role in various neurological disorders, was higher in multiple regions of the brain. Furthermore, other ASD-related parameters such as blood level epoxides, glutamate and other amino acids in the brain, and gut microbiota were altered in juvenile mice. Though it is clear that maternal glyphosate exposure led to alterations in juvenile offspring, this evidence does not prove that glyphosate exposure causes autism. Rather, that maternal glyphosate exposure in mice can lead to ASD-like behaviors and changes in biochemical processes that have been linked to ASD.

NON-TARGET IMPACTS

MAMMALS

Alarcon, R. *et al.* Neonatal exposure to a glyphosate-based herbicide alters the uterine differentiation of prepubertal ewe lambs. *ENVIRONMENTAL POLLUTION* 265, (2020).

Alarcon et al. (2020) exposed newborn lambs to Roundup and studied the effects on uterine development. For 14 days after birth, lambs were injected with Roundup at a dose of 2 mg glyphosate/kg body weight per day. After 45 days, uterine cell proliferation, gene expression, and morphology were analyzed. Glyphosate exposure resulted in reduced cell proliferation rates, increased expression of the cell regulators IGFBP-3 and p27, and altered protein expression in various uterine tissues. Uterine morphology was not affected.

Cai, W. *et al.* Low-dose Roundup induces developmental toxicity in bovine preimplantation embryos in vitro. *ENVIRONMENTAL SCIENCE AND POLLUTION RESEARCH* 27, 16451–16459 (2020).

Cai et al. (2020) investigated how Roundup affects bovine embryos by culturing 2-cell embryos with a range of Roundup concentrations. At concentrations of 0.9 ppm and above, they observed a decrease in blastocyst development and an increase in calcium levels, reactive oxygen species, and apoptosis (or cell death), all of which affect the viability of the embryo. For cattle, exposure to glyphosate is most likely to occur as a result of residues in feed, which could be anywhere from 1 to 344 ppm.

Krause, J. L. *et al.* The glyphosate formulation Roundup (R) LB plus influences the global metabolome of pig gut microbiota in vitro. *SCIENCE OF THE TOTAL ENVIRONMENT* 745, (2020).

Krause et al. (2020) studied the in vitro effects of Roundup on species composition of pig gut microbial communities. Microbes were exposed to Roundup at a rate of 228 g glyphosate/day for three days. This concentration represents the maximum daily intake of glyphosate for pigs as determined by the European Food Safety Authority. Roundup had no effect on pig gut microbiota.

Nerozzi, C. *et al.* Effects of Roundup and its main component, glyphosate, upon mammalian sperm function and survival. *SCIENTIFIC REPORTS* 10, (2020).

Nerozzi et al. (2020) exposed pig sperm to high doses of pure glyphosate or Roundup and investigated the effect on motility, viability, acrosome integrity, mitochondrial activity, and DNA fragmentation. They found that all functional parameters except DNA fragmentation were negatively affected at a range of Roundup concentrations sometimes as low as 5 µg/L, but only at high concentrations (360 µg/mL) of pure glyphosate. Additionally, Roundup was shown to have a concentration-dependent impact on sperm function and survival.

Qiu, S. *et al.* Toxic effects of glyphosate on intestinal morphology, antioxidant capacity and barrier function in weaned piglets. *ECOTOXICOLOGY AND ENVIRONMENTAL SAFETY* 187, (2020).

Qiu et al. (2020) fed piglets Roundup-contaminated food containing 10, 20, or 40 mg glyphosate/kg body weight for 35 days and evaluated the effects on intestinal morphology, immune response, and enzyme activity. Roundup had no effects on morphology or cytokine levels. It did have some effect on gene expression related to oxidative stress and altered antioxidant enzyme activity, but only in the duodenum and at high concentrations. CAT was upregulated at concentrations above 20 mg/kg and SOD and MDA were upregulated at 40 mg/kg.

Schnabel, K. *et al.* Functionality and DNA-damage properties of blood cells in lactating cows exposed to glyphosate contaminated feed at different feed energy levels. *ARCHIVES OF ANIMAL NUTRITION* 74, 87–106 (2020).

Schnabel et al. (2020) studied the influence of two Roundup concentrations (112 and 132 µg glyphosate/kg body weight) on hematological, enzymatic, and genotoxic parameters in dairy cows. They report no effects of Roundup on measured parameters.

Spinaci, M., Nerozzi, C., lo Tamanini, C., Bucci, D. & Galeati, G. Glyphosate and its formulation Roundup impair pig oocyte maturation. *SCIENTIFIC REPORTS* 10, (2020).

Spinaci et al. (2020) compared the effects of Roundup and pure glyphosate on the maturation, development, and enzymatic activity of pig ovarian cells (oocytes). Glyphosate and Roundup had no effects on pig oocyte maturation. However, post fertilization, a reduction in blastocyst development was observed at concentrations above 200 µg/mL glyphosate. This effect was observed for Roundup as well, which also caused a reduction in progesterone levels and an increase in reactive oxygen species at concentrations of 100-360 µg glyphosate/mL and 360 µg glyphosate/mL, respectively.

BIRDS

Fathi, M. A. *et al.* Disruption of cytochrome P450 enzymes in the liver and small intestine in chicken embryos in ovo exposed to glyphosate. *ENVIRONMENTAL SCIENCE AND POLLUTION RESEARCH* 27, 16865–16875 (2020).

Fathi et al. (2020) investigated how exposure to glyphosate and Roundup during embryonic development alters gene expression, enzyme activity, and morphology of the liver and small intestine in chickens. Eggs were exposed, via injection into the air sac, to 10 mg/kg egg mass of pure glyphosate or glyphosate in Roundup; after 15 days tissues were sampled. They found no effect on total embryo weight, but liver weight increased in the Roundup treatment. In the liver, Roundup altered the activity of T-SOD, GST-Px, and MDA, whereas glyphosate only altered GST-Px. Effects of Roundup were similar in the small intestine and, again, glyphosate impacted only one enzyme, T-SOD. Additionally, changes in cytokine mRNA expression were observed for both glyphosate and Roundup. Finally, they report negative effects of glyphosate and Roundup on the morphology of the small intestine. However, it is unclear how these effects could manifest in hatched chicks.

Ruuskanen, S. *et al.* Glyphosate-based herbicides influence antioxidants, reproductive hormones and gut microbiome but not reproduction: A long-term experiment in an avian model. *ENVIRONMENTAL POLLUTION* 266, (2020).

In this long-term study, Ruuskanen et al. (2020a) orally exposed quails (*Coturnix japonica*) to Roundup for the first year of life. Birds were consistently fed Roundup at a dose of 160 mg glyphosate/kg feed for 52 weeks and a variety of health, reproductive, enzymatic, and gut microbial parameters were measured. They report no effect of Roundup on total body mass, relative organ mass, or reproduction. Of the measured oxidative status biomarkers, only liver catalase activity was altered by Roundup; brain AChE activity was unaffected. In the gut, they observed an increase in overall richness and abundance of bacteria. However, shifts in gut microbial community composition were observed at 12 weeks (particularly in females), but not 28 weeks.

Ruuskanen, S., Rainio, M. J., Uusitalo, M., Saikkonen, K. & Helander, M. Effects of parental exposure to glyphosate-based herbicides on embryonic development and oxidative status: a long-term experiment in a bird model. *SCIENTIFIC REPORTS* 10, (2020).

Ruuskanen et al. (2020b) exposed quails (*Coturnix japonica*) to Roundup at a dose of 160 mg glyphosate/kg feed for 52 weeks in order to assess how parental exposure to glyphosate effects embryo development and physiology. Parental Roundup exposure had no effect on egg/yolk/shell mass, embryo brain mass, egg prenatal thyroid hormones, or antioxidant enzyme activity.

REPTILES

Mestre, A. P. *et al.* Effects of glyphosate, cypermethrin, and chlorpyrifos on hematological parameters of the tegu lizard (Salvator merianae) in different embryo stages. *CHEMOSPHERE* 252, (2020).

Mestre et al. (2020) topically exposed tegu lizard (*Salvator merianae*) eggs to three different pesticides at 3-5 days and 33 days of embryo development then examined blood/immune responses. Direct application of pesticides to eggs is unlikely in the wild because eggs are usually concealed in the nest. Pesticide exposure had no effect on total white blood cell count, regardless of developmental stage. However, for 33-day old eggs exposed to glyphosate (in Roundup) at 400 µg/egg, there was a shift in the types of white blood cells present that was not observed for chlorpyrifos or cypermethrin. Additionally, there was a decrease in neutralizing antibodies for 5-day old eggs exposed to glyphosate (Panzer Gold) at 800 µg/egg.

BEES

Almasri, H. *et al.* Mixtures of an insecticide, a fungicide and a herbicide induce high toxicities and systemic physiological disturbances in winter Apis mellifera honey bees. *ECOTOXICOLOGY AND ENVIRONMENTAL SAFETY* 203, (2020).

Almasri et al. (2020) studied the individual and combined effects of imidacloprid (insecticide), difenoconazole (fungicide), and glyphosate (herbicide) on winter honeybee (*Apis mellifera*) survival and physiology. Bees were exposed to concentrations of 0.1, 1, and 10 µg/L of both individual and combined mixtures of pesticides for 20 days. These concentrations are within the range of doses that bees may be exposed to in the wild as a result of foraging on contaminated nectar or pollen. Survival, food consumption, and activity of the enzymes carboxylesterase para (CaE-3), glutathione-*S*-transferase (GST), acetylcholinesterase (AChE), glucose-6-phosphate dehydrogenase (G6PDH), and alkaline phosphatase (ALP) was measured. Bees exposed to glyphosate generally experienced lower survival rates and higher food consumption. Surviving bees had varied, but minimal, modulation of measured enzymes. A similar trend was observed in other pesticide treatments and effects were most notable in treatments with mixtures of two or three pesticides.

Chaves, A., Faita, M. R., Ferreira, B. L., Poltronieri, A. S. & Nodari, R. O. Effects of glyphosate-based herbicide on royal jelly production of Apis mellifera (Hymenoptera: Apidae) in field conditions. *JOURNAL OF APICULTURAL RESEARCH* 60, 277–279 (2020).

In this study, Chaves et al. (2020) measured how the production of royal jelly (sustenance for hive larvae) in honeybee (*Apis mellifera*) is affected by exposure to Roundup. Hives were fed 6.68 mg/L of glyphosate once a week for one month and royal jelly was collected every 72 hours. They found that royal jelly production was reduced by up to 47.67%. However, the number of hive cells containing larvae did not differ. The likelihood of bees being exposed to this level of glyphosate for a prolonged period of time is minimal.

Delkash-Roudsari, S. *et al.* Assessment of lethal and sublethal effects of imidacloprid, ethion, and glyphosate on aversive conditioning, motility, and lifespan in honey bees (Apis mellifera L.). *ECOTOXICOLOGY AND ENVIRONMENTAL SAFETY* 204, (2020).

Delkash-Roudsari et al. (2020) studied aversive conditioning, mobility, and survival in honeybees exposed to three pesticides. Roundup and glyphosate exposure concentrations ranged from 0.12 to 24 mg/L. Imidacloprid and ethion had negative effects on learning and mobility. Glyphosate had minimal effects on learning but did alter activity. The majority of Roundup concentrations reduced day and night activity (circadian rhythm); there were some effects of glyphosate, but they were unpredictable. Both formulas had varying effects on average activity that was unrelated to dosage. **Survival was unaffected**.

Faita, M. R., Cardozo, M. M., Amandio, D. T. T., Orth, A. I. & Nodari, R. O. Glyphosate-based herbicides and Nosema sp. microsporidia reduce honey bee (Apis mellifera L.) survivability under laboratory conditions. *JOURNAL OF APICULTURAL RESEARCH* 59, 332–342 (2020).

To understand how glyphosate affects bee survival and susceptibility to infection, Faita et al. (2020) exposed adult workers of honeybees (*Apis mellifera*) to Roundup (2.16 µg glyphosate/g artificial food), *Nosema sp.* spores, and *Nosema* + Roundup for 120 hours. *Nosema sp.* is a microsporidium known to cause nosemosis, a disease that weakens bees via decreased protein levels and altered hemolymph composition. **The authors found that neither Roundup nor *Nosema sp.* had a significant effect on bee survival or food consumption. However, when exposed in combination, *Nosema* spp. and Roundup increased food consumption and reduced survival by at least 20%.** These results suggest that exposure to Roundup can exacerbate the symptoms/impacts of pathogen infection even at sublethal doses.

Motta, E. V. S. *et al.* Oral or Topical Exposure to Glyphosate in Herbicide Formulation Impacts the Gut Microbiota and Survival Rates of Honey Bees. *APPLIED AND ENVIRONMENTAL MICROBIOLOGY* 86, (2020).

Throughout a series of lab and field experiments, Motta et al. (2020) investigated the ways in which glyphosate and Roundup alter hive recovery, mortality, gut microbiomes, and sensitivity to *Serratia* infection in honeybees (*Apis melifera*). When bees were orally exposed to 0.1% Roundup in the lab, gut microbial abundance was reduced, specifically of *Snodgrassella alvi*, and the number of bees that returned to the hive dropped by roughly 16% in the majority of trials. Similar effects on gut microbiota were also observed in the field. **Additionally, a challenge experiment revealed that dual exposure to the pathogenic bacteria *Serratia marcescens* and 0.1% Roundup increased mortality.** Researchers also report that glyphosate was detected in the honey of treatment hives at 800-1600 µg/mL when bees were fed 0.1% Roundup. Topical exposure also had an impact. **Bees that were topically exposed at 0.5%, 1%, and 3% Roundup experienced increased mortality**, reduced hive return rates, and reduced abundance of *Snodgrassella alvi*.

Motta, E. V. S. & Moran, N. A. Impact of Glyphosate on the Honey Bee Gut Microbiota: Effects of Intensity, Duration, and Timing of Exposure. *MSYSTEMS* 5, (2020).

Motta & Moran (2020) exposed young honeybees with and without established gut microbiomes to a range of glyphosate concentrations (1.691 mg/L - 169.1 mg/L) for up to 40 days and monitored the response of bee gut microbiota. For bees with and without established microbiota, overall bacterial abundance was minimally affected, but abundance of *Snodgrassella alvi*, an important member of the bee gut microbiome, decreased in a dose-dependent manner. Gut community composition was altered at intermediate and high concentrations and **bee survival decreased at concentrations above 16.91 mg/L. Concentrations of this magnitude can occur in pollen and nectar of plants recently treated with glyphosate, but it is unlikely that bees would be chronically exposed because the herbicide would kill the plant shortly after application.**

Odemer, R. *et al.* Chronic High Glyphosate Exposure Delays Individual Worker Bee (Apis mellifera L.) Development under Field Conditions. *INSECTS* 11, (2020).

Odemer et al. (2020) studied honeybee (*Apis mellifera*) colony response to low (4.8 mg glyphosate/kg) and high (137.6 mg glyphosate/kg) doses of glyphosate-based herbicides (GBH). In experimental conditions, colony health was generally unaffected but there was an increase in brood termination rate and decreased hatching weight after chronic (21 days) high dose exposure. **In the field they found no significant effects of GBH on survival, colony health, or colony conditions during overwintering.**

Strobl, V. *et al.* Positive Correlation between Pesticide Consumption and Longevity in Solitary Bees: Are We Overlooking Fitness Trade-Offs? *INSECTS* 11, (2020).

Strobl et al. (2020) fed adult mason bees (*Osmia bicornis*) to 1.5 ng/g of clothianidin, 10 mg/g of glyphosate, or a mixture of both for up to 70 days and observed changes in survival and feeding behavior. **They found no effects of the pesticides on daily consumption, longevity, or cumulative survival.**

Vazquez, D. E. *et al.* Sleep in honey bees is affected by the herbicide glyphosate. *SCIENTIFIC REPORTS* 10, (2020).

Honeybee (*Apis mellifera*) sleep was monitored after oral exposure to 0, 25, 50, or 100 ng of glyphosate. Vazquez et al. (2020a) found that glyphosate did not significantly affect time spent sleeping or frequency of honeybee sleep . However, at 50 ng of glyphosate antennal activity lessened, indicating that sleep may have deepened at that dose.

Vazquez, D. E., Latorre-Estivalis, J. M., Ons, S. & Farina, W. M. Chronic exposure to glyphosate induces transcriptional changes in honey bee larva: A toxicogenomic study. *ENVIRONMENTAL POLLUTION* 261, (2020).

Vazquez et al. (2020b) chronically exposed honeybees (*Apis mellifera*) larva to glyphosate at concentrations of 2.5 mg/L of food for 120 hours in order to evaluate toxicity in the absence of physical indicators. They report similar larval death rates between the exposed and unexposed groups. However, **there was an 18% increase in the proportion of larva with delayed development in the glyphosate exposure group.** Additionally, in the larva that were exposed but showed no signs of developmental delays, gene expression was altered in a small percentage (0.22%) of sampled protein-coding genes mostly concerned with response to environmental stressors and metabolism.

TERRESTRIAL INVERTEBRATES

Alhewairini, S. S. TOXICITY EFFECTS OF GLYPHOSATE AND METRIBUZIN ON FIVE SPECIES OF SOIL-DWELLING PREDATORY MITES. *PAKISTAN JOURNAL OF AGRICULTURAL SCIENCES* 57, 1429–1435 (2020).

Alhewairini (2020) investigated the effects of two herbicides (glyphosate and metribuzin) on soil mites. Mites were directly sprayed with 0.5x, 1x, and 2x of the recommended field doses of the glyphosate-based herbicide (1.1L/100L) and the metribuzin-based herbicide (0.5kg/ha) and mortality was recorded. **They found that mortality rates of all five species of mites increased in a dose-dependent manner** for both herbicides, with the 2x dose usually resulting in 100% mortality. No comparison was made between the herbicides.

Bataillard, D., Christe, P. & Pigeault, R. Impact of field-realistic doses of glyphosate and nutritional stress on mosquito life history traits and susceptibility to malaria parasite infection. *ECOLOGY AND EVOLUTION* 10, 5079–5088 (2020).

Bataillard et al. (2020) orally exposed mosquito (*Culex pipiens*) larva to pure glyphosate and a glyphosate-based herbicide at 0.05 and 0.10 mg glyphosate/L and observed effects on health and malaria (*Plasmodium relictum*) infection. They found no effects of glyphosate or glyphosate-based herbicide on larval development time, adult mosquito size, adult feeding behavior, number of eggs laid, or severity of malaria infection. There was an increase in larval mortality for mosquitoes exposed to 0.05 mg glyphosate/L, but not 0.10 mg/L, for both pure and formulation glyphosate. They also investigated how glyphosate exposure combined with food stress impacted the same parameters. Under standard food conditions, exposure to 0.05mg/L of pure glyphosate reduced the prevalence, but not intensity, of malaria infection.

Bednarova, A., Kropf, M. & Krishnan, N. The surfactant polyethoxylated tallowamine (POEA) reduces lifespan and inhibits fecundity in Drosophila melanogaster- In vivo and in vitro study. *ECOTOXICOLOGY AND ENVIRONMENTAL SAFETY* 188, (2020).

Bednarova et al. (2020) investigated how lethal and sub-lethal exposure to Roundup and its various chemical components affect health and fecundity of fruit flies (*Drosophila melanogaster)*. Flies were exposed to pure glyphosate (100µg/mL), polyethoxylated tallow amine (POEA; 45µg/mL), and Roundup (15µg/mL) for 24 hours. **Roundup and POEA similarly reduced survival**, impaired negative geotaxis, reduced fecundity and cell viability, and increased carbonylated protein levels; median survival in the treatment group was up to 21 days shorter than the control group and females were laying about 1/3 of the amount of eggs compared to the control. Pure glyphosate had no effect.

Frizzi, F. *et al.* Palatability of glyphosate in ants: a field experiment reveals broad acceptance of highly polluted solutions in a Mediterranean ant. *ENVIRONMENTAL SCIENCE AND POLLUTION RESEARCH* 27, 29666–29671 (2020).

Frizzi et al. (2020) studied how the presence of Roundup affects feeding preferences of ants (*Crematogaster scutellaris*). When presented with four solutions of Roundup at concentrations of 0.036, 0.36, 3.6, 36 g glyphosate/L, ants avoided solutions in a concentration dependent manner. Although the frequency of acceptance was different for all treatments, it remained greater than 60% for all concentrations except 36 g glyphosate/L, when the average acceptance rate was 0%.

Gomez-Gallego, C. *et al.* Glyphosate-based herbicide affects the composition of microbes associated with Colorado potato beetle (Leptinotarsa decemlineata). *FEMS MICROBIOLOGY LETTERS* 367, (2020).

Gomez-Gallego et al. (2020) reared potato beetles *(Leptinotarsa decemlineata)* in soil contaminated with Roundup and assessed gut microbial communities at both the larval and adult stage. Beetles were introduced to plants grown in soil treated with 6.4 L/ha of Roundup. Roundup exposure did not significantly affect microbial diversity or richness at either stage but did affect relative abundance.

Lescano, M. R., Masin, C. E., Rodriguez, A. R., Godoy, J. L. & Zalazar, C. S. Earthworms to improve glyphosate degradation in biobeds. *ENVIRONMENTAL SCIENCE AND POLLUTION RESEARCH* 27, 27023–27031 (2020).

Lescano et al. (2020) evaluated earthworms’ (*Eisenia fetida*) ability to remove glyphosate and its degradation product (AMPA) from soils. They applied 1000 mg glyphosate/kg to four soil mixtures with and without earthworms. **Earthworms expedited glyphosate degradation in all soil mixtures and suffered mortality rates no higher than 13%.** Number of cocoons and juveniles varied across soils. Due to lack of an unsprayed control soil, it is impossible to say whether or not earthworms survival and reproduction is significantly altered by glyphosate.

Maderthaner, M. *et al.* Commercial glyphosate-based herbicides effects on springtails (Collembola) differ from those of their respective active ingredients and vary with soil organic matter content. *ENVIRONMENTAL SCIENCE AND POLLUTION RESEARCH* 27, 17280–17289 (2020).

Maderthaner et al. (2020) measured springtail (*Sminthurinus niger*) activity in soils contaminated with pure glyphosate and glyphosate-based herbicides (GBH), accounting for any influence that soil organic matter may have. They found that pure glyphosate and GBHs at doses ranging from

2.18-2.43 kg/ha increased activity of springtails in soils with high organic matter content but not in soils with low organic matter content. Litter decomposition rate was not affected.

Nuutinen, V. *et al.* Glyphosate spraying and earthworm Lumbricus terrestris L. activity: Evaluating short-term impact in a glasshouse experiment simulating cereal post-harvest. *EUROPEAN JOURNAL OF SOIL BIOLOGY* 96, (2020).

Nuutinen et al. (2020) investigated how glyphosate exposure impacts earthworm (*Lumbricus terrestris*) health and reproduction. Soil was collected from a cereal field, inoculated with earthworms, and sprayed with a glyphosate-based herbicide at a concentration of 0.52 mg/kg of soil. After 61 days, they observed no effect on worm mass, number of cocoons, or incorporation of plant matter into the soil.

Owagboriaye, F. *et al.* Biochemical response and vermiremediation assessment of three earthworm species (Alma millsoni, Eudrilus eugeniae and Libyodrilus violaceus) in soil contaminated with a glyphosate-based herbicide. *ECOLOGICAL INDICATORS* 108, (2020).

Owagboriaye et al. (2020) exposed three species of African earthworms (*Alma millsoni, Eudrilus eugeniae and Libyodrilus violaceus*) to Roundup and observed the effects on enzyme activity and oxidative biomarkers. Worms were indirectly exposed to Roundup at concentrations of 83.2 g glyphosate/m^2 for 48 hours, then monitored for 8 weeks. Roundup did not affect AChE activity, but did alter activity of LDH, MT, and GST. The oxidative biomarkers CAT, SOD, and GPX were altered in all earthworm species; only *E. eugeniae* had modulated GSH.

Pereira, J. L. *et al.* GLYPHOSATE IMPACT ON ARTHROPODS ASSOCIATED TO ROUNDUP READY AND CONVENTIONAL SOYBEAN (Glycine max L.). *PLANTA DANINHA* 38, (2020).

Pereira et al. (2020) compared the effects of mechanical weeding and glyphosate application on South American arthropod communities in transgenic and conventional soybeans. They found no effects of mechanical weeding or transgenic soybeans on arthropod communities. After application of 1,080 g/ha of glyphosate, densities of *Solenopsis* sp. (predator) and *Hypogastrura* sp. (herbivore) decreased, while densities of *Bemisia tabaci*, *Caliothrips brasiliensis*, *Cerotoma arcuatus*, and *Tetranychus* sp.(phytophages) increased.

Pochron, S. *et al.* Glyphosate but not Roundup harms earthworms (Eisenia fetida). *CHEMOSPHERE* 241, (2020).

Pochron et al. (2020) investigated how glyphosate affects earthworm health and soil microbes. They exposed earthworms (*Eisenia fetida*) to soil contaminated with pure glyphosate or one of two Roundup formulations at concentrations of 26.3 mg glyphosate/kg soil for 40 days. Glyphosate, but not Roundup, reduced worm biomass and stress-test survival time. Microbial and fungal biomass were not affected.

Portilla, M. A. & Lawler, S. P. Herbicide treatment alters the effects of water hyacinth on larval mosquito abundance. *JOURNAL OF VECTOR ECOLOGY* 45, 69–81 (2020).

Portilla & Lawler (2020) investigated how mosquito larva (*Culex pipens*) respond to Roundup application in the presence of invasive water hyacinth (*Eichhornia crassipes*). Mosquitoes were exposed to one of four treatments: pure water with and without Roundup and adjuvant and water hyacinth with and without Roundup and adjuvant, which were applied at concentrations of 0.6 mL glyphosate/m^2. They found that larval abundance was reduced after herbicide application in both treatments. In the weeks following application, the number of larvae gradually increased in the water hyacinth treatment, likely due to the availability of decaying plant matter, whereas the open water treatment never recovered.

Rainio, M. J. *et al.* Glyphosate-based herbicide has soil-mediated effects on potato glycoalkaloids and oxidative status of a potato pest. *CHEMOSPHERE* 258, (2020).

Rainio et al. (2020) exposed potato beetles (*Leptinotarsa decemlineata*) to potato plants grown in Roundup-treated soil (6.4 L/ha) and measured any effects on survival and oxidative stress. **Larval and adult survival and body mass were not affected by exposure**. Glutathione (tGSH) concentration and catalase (CAT) activity was altered in larva and adults; superoxide dismutase (SOD) was only altered in larva.

Samal, S., Mishra, C. S. K. & Sahoo, S. Dermal, histological anomalies with variations in enzyme activities of the earthworms Lampito mauritii and Drawida willsi after short term exposure to organophosphate pesticides. *ISJ-INVERTEBRATE SURVIVAL JOURNAL* 17, 117–128 (2020)

Samal et al. (2020) exposed two species of tropical earthworms (*Drawida willsi* and *Lampito mauritii*) to three concentrations of glyphosate (0.1, 0.15, 0.2 g/kg) and the pesticide monocrotophos (1.0, 2.0, 3.0 g/kg) for 24 hours and monitored protein levels, lipid peroxidation (LPX), and enzyme activity. Glyphosate had differing effects depending on the worm species and the concentration, but in general resulted in some alteration of tissue protein levels, LPX, and LDH, AChE, and CAT activity. They also monitored dermal and muscular morphology, but no statistical analyses were used to assess these changes and therefore cannot be interpreted.

TERRESTRIAL MICROORGANISMS

Allegrini, M., Gomez, E. & Zabaloy, M. C. Acute glyphosate exposure does not condition the response of microbial communities to a dry-rewetting disturbance in a soil with a long history of glyphosate-based herbicides. *SOIL* 6, 291–297 (2020).

Allegrini et al. (2020) studied how glyphosate exposure affects microbial resilience to soil dry-out and subsequent rewetting. They applied 49 µg glyphosate/g soil of Roundup to field soil historically treated with glyphosate-based herbicides, then dried and rewet the soil; respiratory response and DNA analysis of microbial communities were performed. They found increased respiratory quotient values after exposure to Roundup, but number of gene copies was not affected. There was no relationship between herbicide application and dry-rewetting response.

Bottrill, D. *et al.* Short-term application of mulch, roundup and organic herbicides did not affect soil microbial biomass or bacterial and fungal diversity. *CHEMOSPHERE* 244, (2020).

Bottrill et al. (2020) compared the effects of mulching versus herbicide application on soil chemistry and microbial communities. Three applications of Roundup (10mL/L) and four applications of acetic acid (125mL/L), BioWeed (200mL/L), and Slasher (700mL/L) were applied at four-month intervals; soil parameters were measured 2 and 8 months after application. They report no effects of mulch or herbicides on pH, C:N ratio, or fungal/bacterial community structure.

Goncalves, O. S., Rodrigues da Silva, M. de F. & Martins, P. F. Glyphosate-induced impact on the functional traits of the Bacillus sp. FC1 isolate. *PESQUISA AGROPECUARIA TROPICAL* 50, (2020).

Goncalves et al. (2020) investigated how Roundup effects the soil bacterium *Bacillus* sp. and its interaction with the pathogenic fungus *Fusarium* sp. Bacteria were exposed to 7.2 mg/mL of glyphosate for 168 hours maximum before measuring colony health and its inhibitory effect on *Fusarium*. They found that bacterial growth was reduced in the presence of glyphosate, but the interaction with *Fusarium* sp. was unaffected.

Gornish, E. S., Franklin, K., Rowe, J. & Barberan, A. Buffelgrass invasion and glyphosate effects on desert soil microbiome communities. *BIOLOGICAL INVASIONS* vol. 22 2587–2597 (2020).

Gornish et al. (2020) compared soil microbiomes among three buffelgrass-invasion (*Cenchrus ciliaris*) sites: uninvaded, invaded, and invaded and treated with glyphosate. Sites were sprayed with one of three glyphosate-based herbicides (Razor Pro, Kleenup Pro, and Ranger Pro) mixed at

3-5%. The analysis of the soil samples showed that glyphosate treatment did not affect bacterial and archaeal richness/evenness or fungal richness/evenness. Bacterial and fungal community composition and functional groups were similar but distinct between treated and untreated sites. However, buffelgrass invasion was the primary driver of soil microbial dynamics.

Kepler, R. M. *et al.* Soil Microbial Communities in Diverse Agroecosystems Exposed to the Herbicide Glyphosate. *APPLIED AND ENVIRONMENTAL MICROBIOLOGY* 86, (2020).

Kepler et al. (2020) studied how farming practices and glyphosate use affect the soil microbiome. Using both conventional and organic farming practices, with and without a history of glyphosate use, they applied glyphosate at a rate of 0.87 kg/ha two times before collecting soil samples. They measured fungal and prokaryotic community structure, *Fusarium* sp. abundance, and crop yields. Glyphosate had no effect on these parameters.

Korzeniewicz, R., Baranowska, M., Kwasna, H., Niedbala, G. & Behnke-Borowczyk, J. Communities of Fungi in Black Cherry Stumps and Effects of Herbicide. *PLANTS-BASEL* 9, (2020).

Korzeniewicz et al. (2020) investigated how glyphosate application altered fungal communities of *Prunus serotina*. Stumps of various sizes were sprayed at rate of 6 L/ha in May and August. Fungal abundance and species richness were altered, but in an unpredictable manner; the direction of change varied depending on time of year and stump size, so in some cases abundance/richness increased, but in others it decreased. In August, glyphosate treatment caused an increase in abundance, whereas in May treatment reduced abundance.

Ramirez-Villacis, D. X. *et al.* Root Microbiome Modulates Plant Growth Promotion Induced by Low Doses of Glyphosate. *MSPHERE* 5, (2020).

Ramirez-Villacis et al. (2020) examined the hormetic effects of low doses of glyphosate and how the root microbiome may influence these effects. *Arabidopsis* seedlings with and without a microbial inoculant were treated with 3.6 µg/L of glyphosate for 12 days. They monitored plant growth in addition to changes in the microbial community and found that glyphosate caused a slight increase in shoot growth in the uninoculated treatment, but had the opposite effect in the inoculated treatment. Root growth was also reduced in the presence of glyphosate and the microbial inoculant, which the authors attribute to a small shift in community composition resulting in enriched root-growth inhibiting microbial strains. However, the mechanism of inhibition remains unclear.

Wilkes, T., I., Warner, D. J., Davies, K. G. & Edmonds-Brown, V. Tillage, Glyphosate and Beneficial Arbuscular Mycorrhizal Fungi: Optimising Crop Management for Plant-Fungal Symbiosis. *AGRICULTURE-BASEL* 10, (2020).

Wilkes et al. (2020) analyzed how glyphosate exposure and tillage alters arbuscular mycorrhizal (AM) fungal symbiosis in wheat plants. Using soil from two farms, one that tills and does not use glyphosate and another that doesn't till and does use glyphosate, they quantified fungal structures and biomass in the field and in the greenhouse. Glyphosate was applied once at 1080 g/ha in the field and at rates of 0-350 g/L in the greenhouse. There were a greater number of AM root structures in no-till soil in the greenhouse but these effects were not observed in the field. Glyphosate application resulted in reduced biomass in both tillage treatments in the greenhouse and in the field.

FISH

Aminov, A., I. & Golovanova, I. L. Effect of Roundup on the Activities of Glycosidase in the Intestines of Typical and Facultative Ichthyophages as a Function of Temperature and pH. *INLAND WATER BIOLOGY* 13, 291–296 (2020).

Exposed intestinal mucosa and chyme of common perch, burbot, pike, zander, and wels catfish (in vitro) to Roundup (25 µg/L) for 1 hour at different temperatures and pH, then measured the activity of the digestive enzyme amylase. Aminov et al. (2020) found that Roundup can have inhibitory effects on amylolytic activity for all species, but sensitivity to these effects is dependent on temperature and pH. Inhibition was typically most severe at acidic pH values. These results suggest Roundup could affect the digestive process in some fish.

Fabian Bonifacio, A., Jimena Zambrano, M. & Cecilia Hued, A. Integrated ecotoxicological assessment of the complex interactions between chlorpyrifos and glyphosate on a non-target species Cnesterodon decemmaculatus (Jenyns, 1842). *CHEMOSPHERE* 261, (2020).

Bonifacio et al. (2020) studied how exposure to Roundup, the insecticide Clorfox, and mixtures of the two, impact health and physiology of the ten spotted live-bearer (*Cnesterodon decemmaculatus*). They measured changes in speed and mobility, health, and physiological biomarkers including cortisol, lactate dehydrogenase (LDH), creatine phosphokinase (CPK), acetylcholinesterase (AChE), and genotoxicity after 96 hours of exposure. Roundup alone, at a dose of 0.2 and 2 mg/L, had no effect on the measured parameters, except for a reduction in cortisol levels. Clorfox altered locomotion, cortisol levels, and nuclear abnormalities; similar effects were observed in the Clorfox + Roundup mixtures.

Kharat, T. L., Rokade, K. B. & Shejule, K. B. Effect of Roundup 41% (glyphosate) on blood serum biochemical parameters of freshwater fish, Rasbora daniconius. *JOURNAL OF ENVIRONMENTAL BIOLOGY* 41, 222–227 (2020).

Kharat et al. (2020) measured blood biochemical markers of slender rasbora (*Rasbora daniconius*) after 28 days of sub-lethal (0.56 ppm) exposure to Roundup. Exposure to Roundup lead to increased activity of the metabolic enzymes alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT), which is indicative of liver damage. They also observed increased glucose levels and decreased total protein.

Lanzarin, G. A. B., Venancio, C. A. S., Monteiro, S. M. & Felix, L. M. Behavioural toxicity of environmental relevant concentrations of a glyphosate commercial formulation - RoundUp (R) UltraMax - During zebrafish embryogenesis. *CHEMOSPHERE* 253, (2020).

In this study, Lanzarin et al. (2020) observed how exposure to Roundup during embryo development influences zebrafish (*Danio rerio*) behavior and survival. They exposed embryos to Roundup at 1, 2, or 5 µg glyphosate/mL for 72 hours and monitored larval behavior, cortisol levels, and survival. **Roundup had no effect on social or exploratory behavior, hatching rate, cortisol levels, or survival of zebrafish larva.** However, when presented with visual stimulus, the high exposure group spent less time at the bottom half of the enclosure compared to the control. This lack of response to stimulus could make them more vulnerable to predation.

Ma, J. & Li, X. Alteration in the cytokine levels and histopathological damage in common carp induced by glyphosate (vol 128, pg 293, 2015). *CHEMOSPHERE* vol. 245 (2020).

Ma & Li (2020) assessed the effects of sub-acute exposure to glyphosate on organ health and immune response in common carp (*Cyprinus carpio*). After determining the 96 hour LC_{50} to be 520.77 mg/L, they exposed fish to 1/10 and 1/5 the $LC_{50\,96h}$ for 24, 72, and 168 hours and measured cytokine levels in the liver, kidneys, and spleen. They found that proinflammatory cytokine levels were altered in all sampled organs for the majority of concentrations and exposure intervals. They also report physical damage to the liver, kidneys, and spleen, but do not provide any statistical analysis, so it cannot be interpreted.

Menendez-Helman, R. J., Miranda, L. A., Salibian, A. & dos Santos Afonso, M. Effects on Ultrastructure, Composition and Specific Surface Area of the Gills of Odontesthes bonariensis Under Subchronic Glyphosate-Based Herbicide Exposure. *BULLETIN OF ENVIRONMENTAL CONTAMINATION AND TOXICOLOGY* 105, 835–840 (2020).

Menendez-Helman et al. (2020) investigated how glyphosate exposure alters gill morphology in pejerrey fish (*Odontesthes bonariensis*). Fish were exposed to a glyphosate-based herbicide (Glifosato) at 1 or 10 mg glyphosate/L for 15 days. They found no change in the gill elemental composition and an increase in gill specific surface area for fish in the 1 mg glyphosate/L treatment but not for 10 mg glyphosate/L. They also report alterations in gill structure at both concentrations but provide only a qualitative analysis.

Moraes, J. S. *et al.* Zebrafish (Danio rerio) ability to activate ABCC transporters after exposure to glyphosate and its formulation Roundup Transorb (R). *CHEMOSPHERE* 248, (2020).

Moraes et al. (2020) studied the effects of glyphosate on protein activity and gene expression of ABCC transporters in zebrafish (*Danio rerio*). ABC proteins are a group of membrane proteins involved in cellular transport. They exposed fish to 0.1 mg/L of pure glyphosate and glyphosate in Roundup formulation for 24 and 96 hours before measuring protein amounts in the gills, gut, liver, and brain. Glyphosate altered gene expression in the gills, gut, and brain; Roundup altered gene expression in the gills, gut, and liver. Roundup and glyphosate exposure resulted in a similar increase in protein activity in the gills, gut, and liver. Only glyphosate increased protein activity in the brain.

Pompermaier, A. *et al.* Waterborne agrichemicals compromise the anti-predatory behavior of zebrafish. *ENVIRONMENTAL SCIENCE AND POLLUTION RESEARCH* 27, 38559–38567 (2020).

Pompermaier et al. (2020) evaluated how exposure to herbicides containing glyphosate (GBH), methylbenzoate (MBH), or 2,4-Dichlorophenoxyacetic acid (DBH) affects how zebrafish (*Danio rerio*) respond to predatory stimulus. They measured how much time zebrafish spent in the central area, entries into the central area, distance traveled at the periphery, and total distance traveled after the predator simulation. They found that exposure to DBH and GBH at 10% of the LC_{50} (29 µL/L and 1.4 µL/L) respectively, caused fish to spend more time in the central area. GBH also increased the number of entries into the central area. These findings suggest that herbicide exposure may result in increased susceptibility to predation.

Shiry, N., Alavinia, S. J., Gholamhosseini, A. & Mirvaghefi, A. A bioassay on tissue cholinesterase activity of Rutilus kutum (Kamensky, 1901) exposed to some common pesticides in Iran. *VETERINARY RESEARCH FORUM* 11, 325–331 (2020).

Shiry et al. (2020) compared the effects of malathion, carbaryl, and glyphosate on acetylcholinesterase activity in Caspian kutum (*Rutilus kutum*) fries. They first determined the 96-

hour LC_{50} to be 0.97, 11.69, and 6.64 mg/L for malathion, carbaryl, and glyphosate, respectively. Then they measured acetylcholinesterase activity following 15 day exposure to 10% and 20% of the 96-h LC_{50} for each pesticide and found that activity was altered at both doses for malathion and glyphosate, but only at the highest dose for carbaryl.

AMPHIBIANS

Agostini, M. G., Roesler, I., Bonetto, C., Ronco, A. E. & Bilenca, D. Pesticides in the real world: The consequences of GMO-based intensive agriculture on native amphibians. *BIOLOGICAL CONSERVATION* 241, (2020).

This large-scale field study evaluated the in situ effects of pesticide application on tadpole health for four species of South American frogs/toads. Researchers monitored concentrations of endosulfan, cypermethrin, glyphosate, and 2,4-Dichlorophenoxyacetic acid (2,4-D), and tadpole survival and mobility in ponds adjacent to cropland 24 hours before and 48 hours after application of pesticides. Pesticide concentrations ranged anywhere from 0-365 µg/L. **Following application, survival decreased in all ponds except those that contained only glyphosate** and two of the ponds with glyphosate and 2,4-D. For all surviving tadpoles (in all ponds), mobility was greatly reduced. Glyphosate was the had the lowest lethality of the tested pesticides, but had similar effects on mobility.

Bolis, A. et al. Exposure during embryonic development to Roundup(R) Power 2.0 affects lateralization, level of activity and growth, but not defensive behaviour of marsh frog tadpoles. ENVIRONMENTAL POLLUTION 263, (2020).

Bolis et al. (2020) studied how sublethal exposure to Roundup alters growth and activity of tadpoles. Marsh frog (*Pelophylax ridibundus)* embryos were placed in Roundup solutions containing 0.7, 3.1, or 7.6 mg glyphosate/L for 96 hours. Tadpoles were then measured or observed in two sequential behavioral trials. They report an increase in total length at intermediate glyphosate concentrations (0.7 and 3.1 mg/L) and a decrease at the highest concentration (7.6 mg/L), suggesting a possible hormetic effect. Rotational preference and basal activity were negatively impacted in the first behavioral trial, but the effects did not persist to the second trial. Antipredator response was unaffected.

Edge, C. B. *et al.* Compensatory indirect effects of an herbicide on wetland communities. *SCIENCE OF THE TOTAL ENVIRONMENT* 718, (2020).

Edge et al (2020) assessed glyphosate's indirect effect on wetland communities via its direct effect on macrophytes. They show that the combination of direct and indirect effects resulted in a net-zero response at the community level. In a particular example, glyphosate was shown to negatively affect macrophyte cover and wood frog abundance. However, as macrophyte cover decreased, wood frog abundance increased, offsetting the direct effects of glyphosate. This study highlights the importance of examining glyphosate effects within an ecosystem context.

Herek, J. S. *et al.* Can environmental concentrations of glyphosate affect survival and cause malformation in amphibians? Effects from a glyphosate-based herbicide on Physalaemus cuvieri and P. gracilis (Anura: Leptodactylidae). *ENVIRONMENTAL SCIENCE AND POLLUTION RESEARCH* 27, 22619–22630 (2020).

Herek et al. (2020) evaluated the effects of Roundup on the development and survival of two South American frogs. They measured tadpole length, mortality, and malformations after exposure to 65-1000 µg/L for 14 days. The maximum recorded glyphosate concentrations in aquatic environments is 427 µg/L. Glyphosate, in Roundup formulation, had significant effects on all measured parameters for both species. **Mortality increased at concentrations as low as 144 µg glyphosate/L after 14 days of exposure**, though it never exceeded 15%. For those that survived, length and mass decreased at 500 µg glyphosate/L and above, and the severity and frequency of internal and external malformations increased in a concentration-dependent manner.

Leeb, C. *et al.* Avoidance behavior of juvenile common toads (Bufo bufo) in response to surface contamination by different pesticides. *PLOS ONE* 15, (2020).

This study tested whether or not toads will physically avoid surfaces contaminated with pesticides. Leeb et al. (2020) placed European toads (*Bufo bufo*) in petri dishes with one uncontaminated side and another side contaminated with 10% or 100% of the maximum recommended field rate for each pesticide. For the glyphosate-based herbicide (Taifun Forte) that equated to glyphosate concentrations of 0.18 and 1.8 kg/ha. Frogs were observed in the dishes for 24 hours. They found that avoidance was significant for the glyphosate formulation and two of the fungicide formulations at the maximum concentration, meaning that the toads spent less than 50% of their time on the contaminated side. Additionally, they observed a significant increase in the distance moved per hour on the contaminated side compared to the uncontaminated one. These results suggest that toads may knowingly avoid pesticides, including glyphosate-based herbicides, at high concentrations.

Moutinho, M. F., de Almeida, E. A., Espindola, E. L. G., Daam, M. A. & Schiesari, L. Herbicides employed in sugarcane plantations have lethal and sublethal effects to larval Boana pardalis (Amphibia, Hylidae). *ECOTOXICOLOGY* 29, 1043–1051 (2020).

Moutinho et al. (2020) exposed tree frog (*Boana pardalis*) larva to the maximum recommended concentrations of five herbicides for 23 days then measured performance, behavior, and enzyme activity. Of all the herbicides, 4.0 mg/L of ametryn was the only one that had a significant effect on all measured parameters. Glyphosate, at 2.4 mg/L, also increased mortality rates by 14.9%, but had no effect on final mass, developmental stage, or activity rates; it did result in increased AChE activity, but not GST activity.

Riano, C., Ortiz-Ruiz, M., Rocio Pinto-Sanchez, N. & Gomez-Ramirez, E. Effect of glyphosate (Roundup Active (R)) on liver of tadpoles of the colombian endemic frog Dendropsophus molitor (amphibia: Anura). *CHEMOSPHERE* 250, (2020).

Riano et al. (2020) exposed tadpoles (*Dendropsophus molitor*) to Roundup at concentrations of 325 and 750 µg glyphosate/L for 30 days. Although they measured a variety of histopathological parameters, they only report significant effects of Roundup on number and area of lipid vesicles. They also highlight that this effect is dose-dependent, with a greater concentration resulting in greater number and filled area of lipid vesicles.

Slaby, S. *et al.* Effects of glyphosate and a commercial formulation Roundup (R) exposures on maturation of Xenopus laevis oocytes. *ENVIRONMENTAL SCIENCE AND POLLUTION RESEARCH* 27, 3697–3705 (2020).

Slaby et al. (2020) exposed African clawed frog (*Xenopus laevis*) oocytes to 0.148, 1.48, 14.8, 148, and 1480 µg glyphosate/L of Roundup and glyphosate in vitro. After "overnight" exposure, they analyzed oocyte maturation (induced and not induced), abnormalities, and signaling pathways. When maturation was induced with progesterone, they found that only glyphosate negatively affected the maturation process, with the most pronounced effects occurring at 1480 µg/L. However, in the absence of progesterone, 1480 µg glyphosate/L of Roundup spontaneously induced oocyte maturation. Additionally, only at the highest concentrations of Roundup did they observe increased cytological abnormalities. They observed no effect on signaling pathways for either pure glyphosate or Roundup.

Turhan, D. O., Gungordu, A. & Ozmen, M. Developmental and lethal effects of glyphosate and a glyphosate-based product on Xenopus laevis embryos and tadpoles. *BULLETIN OF ENVIRONMENTAL CONTAMINATION AND TOXICOLOGY* 104, 173–179 (2020).

Turhan et al. (2020) investigated the effects of both pure glyphosate and Roundup on mortality, growth, and enzyme activity of developing African clawed frogs (*Xenopus laevis*). For Roundup, they report a 96 h LC_{50} of 32.1 mg glyphosate/L (embryos) and 35.1 mg glyphosate/L (stage 46 tadpoles), meaning that lethality was 50% at a minimum of 32.1 mg glyphosate/L Roundup. Sub-lethal exposure to Roundup lead to stunted growth and decreased activity of carboxylesterase at 17.6 mg/L. Pure glyphosate had no effect on growth or enzyme activity, and at the highest concentrations (500 mg/L) the maximum lethality was 17% for embryos and tadpoles. The low lethality and limited effects of pure glyphosate compared to Roundup led the authors to conclude that the additives present in Roundup may be responsible for the adverse effects on developing African clawed frogs.

AQUATIC INVERTEBRATES

Asnicar, D., Cappelli, C., Sallehuddin, A. S., Maznan, N. A. & Marin, M. G. Effects of Glyphosate-Based and Derived Products on Sea Urchin Larval Development. *JOURNAL OF MARINE SCIENCE AND ENGINEERING* 8, (2020).

In this study, Asnicar et al. (2020) evaluated how glyphosate, its metabolite (aminomethylphosphonic acid, AMPA), and its chemical formulation (Roundup) alter larval growth and development of sea urchins (*Paracentrotus lividus*). Fertilized urchin eggs of were exposed to the compounds at concentrations of 1, 10, 50, and 100 µg/L for 24 or 48 hours (for Roundup, concentrations refer to amount of active ingredient glyphosate). After exposure, developmental stages, larval growth, and respiration rate were analyzed. They report delayed larval development, increased physical abnormalities, shortened skeletal rod length, and an increased respiration rate as a result of exposure.

Banaee, M. *et al.* Combined effects of exposure to sub-lethal concentration of the insecticide chlorpyrifos and the herbicide glyphosate on the biochemical changes in the freshwater crayfish Pontastacus leptodactylus. *ECOTOXICOLOGY* 29, 1500–1515 (2020).

Banaee et al. (2020) investigated the individual and combined effect of glyphosate and the insecticide chlorpyrifos on physiological and biochemical parameters in crayfish (*Pontastacus leptodactylus)*. Crayfish were exposed to 2.5 and 5 µg/L of chlorpyrifos and/or 0.4 and 0.8 mg/L of glyphosate for 21 days. Glyphosate and chlorpyrifos individually and in combination altered enzyme activity, glucose/protein/cholesterol/triglyceride levels, immune activity, and oxidative biomarkers (TAO, CAT, MDA). They report zero mortality for all treatments.

de Melo, M. S., Nazari, E. M., Rauh Muller, Y. M. & Gismondi, E. Modulation of antioxidant gene expressions by Roundup (R) exposure in the decapod Macrobrachium potiuna. *ECOTOXICOLOGY AND ENVIRONMENTAL SAFETY* 190, (2020).

de Melo et al. (2020a) measured how Roundup exposure alters gene expression of antioxidant proteins (*sod1*, *cat*, *gpx*, *gst*, *txn*, *hsp70*, *hsp90*) in freshwater prawns (*Macrobrachium potiuna*). Male and female prawns were exposed to 0.0065, 0.065, and 0.28 mg glyphosate/L of Roundup for 7-14 days, after which the hepatopancreas was removed and gene expression was quantified. For the majority of measured proteins, expression was up-regulated in females and down-regulated in males, suggesting females may be more resilient to oxidative stress.

de Melo, M. S., Nazari, E. M., Rauh Muller, Y. M. & Gismondi, E. Roundup (R) disrupts chitinolytic enzyme activity and ecdysteroid concentration in Macrobrachium potiuna. *ENVIRONMENTAL SCIENCE AND POLLUTION RESEARCH* 27, 43396–43402 (2020).

de Melo et al. (2020b) investigated how Roundup affects molting in freshwater prawns (*Macrobrachium potiuna*) by monitoring hormones and enzymes involved in the molting process. After exposing male and female prawns to 0.0065, 0.065, and 0.28 mg glyphosate/L of Roundup for 14 days they collected tissue samples and determined chitobiase enzyme activity and 20-hydroxyecdysone (20-HE) hormone levels. Males and females experienced a reduction in enzyme activity after exposure, but only females had reduced hormone levels. These results indicate that Roundup exposure can affect molting physiology, but it is unclear if/how its impacts manifest physically.

Fabrello, J. *et al.* (2020) Molecular and biochemical responses of vitellogenin in the mussel Mytilus galloprovincialis exposed to the glyphosate-based herbicide Roundup (R) Power **2.0. *ENVIRONMENTAL SCIENCE AND POLLUTION RESEARCH* 27, 26543–26553 (2020).**

Fabrello et al. (2020) evaluated Roundup’s ability to act as an endocrine disruptor in Mediterranean mussels (*Mytilus galloprovincialis*) by measuring vitellogenin (an estrogen-regulated lipid transfer protein). They quantified effects directly via vitellogenin gene expression and indirectly via alkali labile phosphate assay (ALP) after 21 days of exposure to 100 and 1000 µg glyphosate/L. The authors found that Roundup had no effect on vitellogenin gene expression in female mussels but had some effect on ALP. However, findings were inconsistent across tissue samples (male/female; gonadal/non-gonadal) and typically occurred after chronic, high exposure. After 21 days, ALP levels decreased in female gonads and increased in male gonads. Non-gonadal ALP was only affected in males after 21 days of exposure at 100 µg/L.

Fanton, N., Bacchetta, C., Rossi, A. & Florencia Gutierrez, M. Effects of a glyphosate-based herbicide on the development and biochemical biomarkers of the freshwater copepod Notodiaptomus carteri (Lowndes, 1934). ***ECOTOXICOLOGY AND ENVIRONMENTAL SAFETY*** **196, (2020).**

Fanton et al. (2020) studied the response of a freshwater copepod (*Notodiaptomus carteri*) to glyphosate-based herbicide exposure. Copepod larva were reared in herbicide treatments consisting of 0.38 or 0.82 mg glyphosate/L for 30 days during which development, morphology, and enzyme activity were recorded. Researchers found that glyphosate exposure disrupted development at low doses and stopped development at higher doses; final body length and caloric content were not affected. Activity of the enzymes GST and SOD increased in females, but not males.

Mottier, A. ***et al.*** **In vitro effects of glyphosate-based herbicides and related adjuvants on primary culture of hemocytes from Haliotis tuberculata.** ***FISH & SHELLFISH IMMUNOLOGY*** **100, 1–8 (2020).**

In an in vitro study, Mottier et al. (2020) measured the response of European abalone (*Haliotis tuberculata*) hemocytes (immune cells) to glyphosate, Roundup, and POEA adjuvants via viability assessment, phagocytic activities, and lysosomal stability. Viability and phagocytic activity were found to be significantly reduced after exposure to Roundup and POEA adjuvant, but not pure glyphosate. Lysosomal stability decreased at low concentrations of glyphosate and high concentrations of Roundup and POEA adjuvant. The authors note that concentrations used in this study (>1000 µg/L) far exceed environmentally realistic concentrations.

Suppa, A. ***et al.*** **Roundup causes embryonic development failure and alters metabolic pathways and gut microbiota functionality in non-target species.** ***MICROBIOME*** **8, (2020).**

Suppa et al. (2020) measured changes in life-history traits, gene expression, and the gut microbiome of the model species *Daphnia magna* following exposure to 1 mg/L of glyphosate and Roundup. They found that both pure glyphosate and Roundup negatively affected growth, maturation, development, and fecundity. Additionally, they report significant genotoxicity and changes in relative abundance and composition of gut microbiota as a result of glyphosate and Roundup exposure. However, aquatic species are unlikely to encounter glyphosate concentrations that exceed 0.5 mg/L even in severe cases of exposure.

Xiang, H., Zhang, Y., Atkinson, D. & Sekar, R. Effects of anthropogenic subsidy and glyphosate on macroinvertebrates in streams. ***ENVIRONMENTAL SCIENCE AND POLLUTION RESEARCH*** **27, 21939–21952 (2020).**

Xiang et al. (2020) investigated how anthropogenic stream inputs, specifically carrion and glyphosate, affect aquatic macroinvertebrates in a variety of land-use types. Leaf litter bags contaminated with chicken meat, Roundup (85 µg glyphosate), or both were placed in four streams for 30 days. They measured invertebrate abundance, richness, and diversity, as well as water quality. **Macroinvertebrate communities differed based on stream location and land use but were not affected by carrion or glyphosate inputs.**

AQUATIC MICROORGANISMS

Alejandra Sabio y Garcia, C. *et al.* New findings on the effect of glyphosate on autotrophic and heterotrophic picoplankton structure: A microcosm approach. *AQUATIC TOXICOLOGY* 222, (2020).

Alejandro Sabio y Garcia et al. (2020) studied how glyphosate exposure affects picoplankton communities in cases of both high and low turbidity. They found that glyphosate had some effect on both autotrophic and heterotrophic picoplankton communities after 8 days of exposure to 4 mg/L glyphosate, a dose which is 10 times greater than realistic worst-case exposure. Effects of glyphosate on heterotrophic picoplankton were similar regardless of turbidity; abundance and richness were not altered after 8 days, but community structure differed based on glyphosate exposure. Abundance of autotrophic picoplankton decreased in response to glyphosate exposure in the high turbidity system but not in low turbidity. However, community structure was not altered by the glyphosate treatment in high turbidity, but was in low turbidity.

Castro Berman, M. *et al.* Field evidence supports former experimental claims on the stimulatory effect of glyphosate on picocyanobacteria communities. *SCIENCE OF THE TOTAL ENVIRONMENT* 701, (2020).

Castro Berman et al. (2020) investigated how picocyanobacteria (Pcy) respond to glyphosate exposure in the field. They compared Pcy abundance and community composition in lakes with and without a decade's long history of agricultural/glyphosate influence. They found that Pcy abundance was much greater in lakes with a history of glyphosate use. However, **they found no relationship between glyphosate concentrations and Pcy abundance**. Farming practices and expression of the phnD gene, which is indicative of the ability to utilize glyphosate and other phosphonates, were used by the authors as glyphosate-impact indicators and explained the variance in Pcy abundances.

de Caralt, S., Verdura, J., Verges, A., Ballesteros, E. & Cebrian, E. Differential effects of pollution on adult and recruits of a canopy-forming alga: implications for population viability under low pollutant levels. *SCIENTIFIC REPORTS* 10, (2020).

de Caralt et al. (2020) explored the effects of four pollutants, nitrate, copper, lead, and glyphosate, on health, fecundity, and recruitment of the macroalga *Carpodesmia crinita*. Adults were exposed to low concentrations of nitrate, copper, and lead, and three concentrations of glyphosate (0.5, 1, and 10 µg/L) for six months. **The authors report no effects of glyphosate on adult health or fecundity. However, they found that exposure to glyphosate at concentrations of 1 and 10 mg/L, resulted in the lowest recruitment density (1.5 individuals/cm^2) and the smallest recruits (size similar in nitrate treatment) compared to all other treatments.** These findings suggest that glyphosate poses no threat to adult macroalga but may have impacts on recruitment success and could negatively affect population dynamics of macroalga.

de Carvalho, R. C. *et al.* Glyphosate-Based Herbicide Toxicophenomics in Marine Diatoms: Impacts on Primary Production and Physiological Fitness. *APPLIED SCIENCES-BASEL* 10, (2020).

de Carvalho et al. (2020) investigated the effects of Roundup on physical and cellular properties of marine diatoms (*Phaeodactylum tricornutum*) after 48 hours of exposure. At glyphosate concentrations of 250 µg/L and above, diatoms experienced an increase in protein and fatty acid content and a decrease in cell density and specific growth rate. Photochemistry and energy balance were negatively affected at high concentrations of glyphosate. Activity of the enzymes CAT and APX did not differ, but SOD activity decreased at 500 µg/L

Fugere, V. *et al.* Community rescue in experimental phytoplankton communities facing severe herbicide pollution. *NATURE ECOLOGY & EVOLUTION* vol. 4 578+ (2020).

In this study, Fugere et al. (2020) examined how phytoplankton communities respond to dangerously high levels of glyphosate and how that response may be mediated by high phytoplankton biomass and glyphosate conditioning. Low- and high-biomass phytoplankton communities were exposed to a range of Roundup concentrations (0-15 mg glyphosate/L) for 45 days (Phase 1) before being treated with 40 mg glyphosate/L Roundup (Phase 2). During this time, they monitored water quality, biomass, and community composition. **Glyphosate exposure in phase 1 had an overall positive effect on biomass and no effect on community composition. Phase 2 also showed a positive relationship between exposure and biomass**, that the authors solely attribute to glyphosate exposure during phase 1. These results suggest that phytoplankton may exhibit some amount of community resilience to glyphosate, also known as community rescue.

Lozano, V. L., Dohle, S. A., Vera, M. S., Torremorell, A. & Pizarro, H. N. Primary production of freshwater microbial communities is affected by a cocktail of herbicides in an outdoor experiment. *ECOTOXICOLOGY AND ENVIRONMENTAL SAFETY* 201, (2020).

Lozano et al. (2020) studied the effects of two herbicides (Roundup and AsiMax) on abundance and productivity of aquatic microbes. Plankton were exposed to 0.3 mg glyphosate/L (low) or 3.0 mg glyphosate/L (high) of Roundup, 0.135 mg 2,4-D/L (low) or 1.35 mg 2,4-D/L (high) of AsiMax, or a mixture at low and high concentrations for 23 days. **At low concentrations, glyphosate had no effect on the abundance of autotrophic picoplankton, heterotrophic bacteria, and micro and nanophytoplankton or the level of chlorophyll a, primary production, and respiration.** 2,4-D and the mixture had some negative effects at low concentrations, but only in the first 24 hours. At high concentrations, the direction of effects fluctuated throughout the study period, but did not persist to day 23 except for an increase in picoplankton abundance in the Roundup treatment and an increase in heterotrophic bacteria abundance in the Roundup treatment and the AsiMax treatment. Roundup was the only substance to have a significant effect on all measured parameters.

Lu, T. *et al.* Understanding the influence of glyphosate on the structure and function of freshwater microbial community in a microcosm. *ENVIRONMENTAL POLLUTION* 260, (2020).

Lu et al. (2020) exposed a freshwater microbiome to 2.5 mg/L of glyphosate for 15 days and measured functional changes and changes in community composition. While they found no effects on community composition, they did report changes in the abundance of coding transcripts related to metabolism, cellular processes, and genetic/environmental information processing. These changes were not unidirectional and appeared to counteract one another. The functional response of this aquatic microbiome suggests that communities similar to this one are capable of withstanding glyphosate-induced stress.

Silva, L. C. M., Daam, M. A. & Gusmao, F. Acclimation alters glyphosate temperature-dependent toxicity: Implications for risk assessment under climate change. *JOURNAL OF HAZARDOUS MATERIALS* 385, (2020).

Silva et al. (2020) assessed how changes in temperature can affect the toxicity of glyphosate in a tropical cladoceran (*Ceriodaphnia silvestrii*). Organisms were acclimated to temperatures of 20˚C, 25˚C, or 30˚C then exposed to a range of glyphosate concentrations (1-400 µg/L) while also being subjected to differing temperatures. They found that when *C. silvestrii* was acclimated at 30˚C mortality rates were less severe. This suggests that in warming waters, the effects of glyphosate are reduced.

PLANTS

Carpenter, D. J. *et al.* Effects of Herbicides on Flowering. *ENVIRONMENTAL TOXICOLOGY AND CHEMISTRY* 39, 1244–1256 (2020).

Carpenter et al. (2020) evaluated the non-target effects of five herbicides, including glyphosate and clopyralid, on flowering intensity and phenology. Nine species of native plants (*Centaurea cyanus, Silene noctiflora, Viola arvensis, Cerastium arvense, Cirsium arvense, Epilobium montanum, Knautia arvensis, Taraxacum officinale,* and *Trifolium pratense*) were sprayed with 1 or 5% of the recommended field rate of each herbicide at the bud stage and the 6- to 8-leaf stage (glyphosate: 14.4 and 72.0 g/ha and clopyralid: 0.8 and 4.0 g/ha). All herbicides caused a reduction in total flower number and a delay in peak flowering time for one or more species, but glyphosate was the most potent. The differences in effects between the bud stage and the 6- to 8-leaf stage were minimal. Alterations in the floral display and phenology of native plants could affect fecundity and pollination success.

Carreiro, M. M., Fuselier, L. C. & Waltman, M. Efficacy and Nontarget Effects of Glyphosate and Two Organic Herbicides for Invasive Woody Vine Control. *NATURAL AREAS JOURNAL* 40, 129–141 (2020).

Carreiro et al. (2020) compared Roundup to the organic herbicides, Scythe and Weed Zap, by studying their effectiveness at controlling invasive plants and their impact on soil organisms, mosses, and ferns. Four species of invasive woody vines were sprayed with either 43.2 kg ai/ha Roundup, 83.8 kg ai/ha Scythe, or 150 kg/ha of Weed Zap and defoliation, nematode presence, moss/fern spores were measured. All herbicides similarly defoliated the woody vines. **None of the herbicides had an effect on nematode abundance or total moss or fern spores.**

Mobli, A., Matloob, A. & Chauhan, B. S. Glyphosate-induced hormesis: impact on seedling growth and reproductive potential of common sowthistle (Sonchus oleraceus). *WEED SCIENCE* 68, 605–611 (2020).

This study investigated how growth and fecundity of the weed *Sonchus oleraceus* is affected by sublethal doses of glyphosate. Seedlings were sprayed with 2.5, 5, 10, 20, 40, 80, or 800 g/ha of glyphosate. Height, number of leaves, average seed count, and biomass were measured. They found that glyphosate significantly affected all traits with the highest recorded values occurring at low doses of glyphosate (specifically 5 g/ha). These findings would suggest that glyphosate can have a hormetic effect on *S. oleraceus*, however further study is necessary to determine if these effects are consistent and applicable in the field.

Russo, L., Buckley, Y. M., Hamilton, H., Kavanagh, M. & Stout, J. C. Low concentrations of fertilizer and herbicide alter plant growth and interactions with flower-visiting insects. *AGRICULTURE ECOSYSTEMS & ENVIRONMENT* 304, (2020).

Russo et al. (2020) examined how plant growth and pollination are altered by non-target herbicide and fertilizer exposure. They applied glyphosate and NPK fertilizer to seven flowering plants (*Cirsium vulgare, Epilobium hirsutum, Plantago lanceolata, Origanum vulgare, Filipendula ulmaria, Hypochaeris radicata,* and *Phacelia tanacetifolia*) at concentrations mimicking non-target exposure in an agricultural setting; the glyphosate-based herbicide was applied once a week for three months totaling 4.4 mg glyphosate/L (about 7% of the field application rate). They found that glyphosate negatively affected flowering height, leaf length, and pollinator visitation. However, when applied in combination with the fertilizer, short-term affects were mediated.

ENVIRONMENTAL FATE

Maggi, F., la Cecilia, D., Tang, F. H. M. & McBratney, A. The global environmental hazard of glyphosate use. *SCIENCE OF THE TOTAL ENVIRONMENT* 717, (2020).

Maggi et al. (2020) employed modeling techniques to describe glyphosate and AMPA distribution and degradation agricultural areas. Glyphosate and AMPA contamination were low but widespread throughout agricultural areas. Residue quantities were largely influenced by soil and environmental variables. They concluded that the risk of environmental exposure to glyphosate or AMPA was low.

Medalie, L. *et al.* Influence of land use and region on glyphosate and aminomethylphosphonic acid in streams in the USA. *SCIENCE OF THE TOTAL ENVIRONMENT* 707, (2020).

Medalie et al. (2020) complied data from 2015-2017 on glyphosate and AMPA concentrations in streams across the US. Using 70 different streams from watersheds of various size and surrounding land-use, they analyzed maximum and mean concentrations, as well as frequency of detection. Using actual sample values, the authors computed hourly estimates to generate a maximum 21-day moving average concentration. They found that glyphosate and AMPA were present in 74% and 90% of samples, respectively. The maximum concentration, using the estimation method previously described, was 6.08 µg/L of glyphosate and 5.07 µg/L of AMPA. Though the authors note limits in their sampling design, this study offers reasonable estimates of environmentally relevant concentrations of glyphosate and its degradation product in waterways.

1.2 *IMAZAPYR*

EFFICACY

Barksdale, N., Byrd, J. D., Zaccaro, M. L. M. & Russell, D. P. Evaluation of herbicide efficacy and application timing for giant miscanthus (Miscanthus x giganteus) biomass reduction. *WEED TECHNOLOGY* 34, 371–376 (2020).

Barksdale et al. (2020) evaluated the effectiveness of 13 herbicides (including glyphosate, imazapyr, and clethodim) in controlling established stands of giant miscanthus (*Miscanthus* x *giganteus*). **They found that applications of glyphosate at 1.3, 2.2, 4.5, and 7.3 kg/ha were most effective in controlling giant Miscanthus and reducing biomass** and that summer is the ideal time for herbicide application. Imazapyr (56, 280, 560 g/ha) and clethodim (56 and 280 g/ha) were similarly ineffective in managing giant miscanthus.

Prince, C. M. & Macdonald, G. E. Chemical control of torpedograss and common reed under altered salinity conditions. *JOURNAL OF AQUATIC PLANT MANAGEMENT* 58, 26–35 (2020).

Prince & Macdonald (2020) investigated how salinity alters the effectiveness of two herbicides (glyphosate and imazapyr) when used to control torpedograss (*Panicum repens*) and common reed (*Phragmites australis*). Plants were grown in either low or high salinity before being sprayed with 0.56, 1.12, 2.24, and 4.48 kg/ha of glyphosate or 0.14, 0.28, 0.56, and 1.12 kg/ha of imazapyr. In the high salinity treatment, torpedograss was less sensitive to low doses of glyphosate and high doses of imazapyr. Because various torpedograss traits, such leaf area, stem number, and others, were reduced in high salinity, the authors suspect that limited herbicide effects were the result of reduced contact area. Common reed was minimally affected by high salinity and the herbicide effects between low and high salinity were also minimal.

Vogt, J. T. *et al.* Efficacy of five herbicide treatments for control of Pyrus calleryana. *INVASIVE PLANT SCIENCE AND MANAGEMENT* 13, 252–257 (2020).

Vogt et al. (2020) applied five herbicide treatments to the woody invasive *Pryus calleryana* to test their efficacy in managing this species. Glyphosate, imazapyr, triclopyr, triclopyr + aminopyralid, and hexazinone were applied at 4 sites. All treatments were similarly effective in killing *P. calleryana*.

1.3 *AMINOPYRALID*

EFFICACY

Aulakh, J. S. Role of nitrogen and herbicides in integrated management of mugwort (Artemisia vulgaris) in cool-season forage grasses. *INVASIVE PLANT SCIENCE AND MANAGEMENT* 13, 189–198 (2020).

Aulakh (2020) compared the effectiveness of three herbicides (aminopyralid, clopyralid, and glyphosate) in controlling invasive mugwort (*Artemisia vulgaris*). Aminopyralid (61, 122, and 244 g/ha), clopyralid (140, 280, and 560 g/ha), and glyphosate (552, 1,104, and 2,208 g/ha) were applied annually to experimental mugwort plots and control, density, and rhizome biomass were monitored for up to 33 months. Mugwort was effectively managed by low doses of aminopyralid. After 33 months, applications of 61 g/ha of aminopyralid resulted in 95% control, 94% reduction in density, and 91% reduction in rhizome biomass. Glyphosate was slightly more effective at doses of 1,104 g/ha and above. In general, higher doses over longer periods of time were most effective. Clopyralid was not useful in managing mugwort. Additionally, they investigated how nitrogen application and herbicide application would influence grass biomass. They found that aminopyralid and clopyralid did not seriously affect grass biomass, but glyphosate application caused a substantial reduction.

Bobadilla, L. K., Hulting, A. G., Curtis, D. W. & Mallory-Smith, C. Application of synthetic auxin herbicides to suppress seed viability of Italian ryegrass (Lolium perenne ssp. multiflorum) in tall fescue seed production. *WEED TECHNOLOGY* 34, 489–497 (2020).

Bobadilla et al. (2020) assessed how the weed *Lolium perenne* ssp. *multiflorum* responded to one of eight auxin herbicide treatments and how those treatments affected non-target tall fescue cultivation. Of the eight treatments, aminopyralid was the only herbicide that reduced seed viability and seed weight of *L. perenne* when it was applied at 0.5 kg/ha. However, aminopyralid also significantly reduced seed viability and weight of tall fescue.

1.4 *TRICLOPYR*

HUMAN HEALTH

Pardo, L. A. *et al.* Pesticide exposure and risk of aggressive prostate cancer among private pesticide applicators. *ENVIRONMENTAL HEALTH* 19, (2020).

Pardo et al. (2020) assessed the relationship between exposure to 39 pesticides, including clethodim, clopyralid, and triclopyr, and prostate cancer. They used available data on private pesticide applicators via the American Health Study to generate hazard ratios. Values greater than 1 with a confidence interval that does not overlap with 1 indicate an elevated risk of prostate cancer when exposed to pesticides. Clethodim and clopyralid had hazard ratios of 1.03 (95%CI 0.64-1.68) and 0.79 (95%CI 0.58-1.08), respectively, suggesting that there is no association between use of these pesticides and prostate cancer. Interestingly, triclopyr had a hazard ratio of 0.68 (95%CI 0.48-0.95) which indicates that the triclopyr applicators included in this analysis had a lower risk of prostate cancer than the general population. This study uses a binary exposure metric, meaning that pesticide exposure was quantified as never used vs. ever used, so the severity or frequency of exposure was not taken into account.

NON-TARGET IMPACTS

Setyawan, Y. P., Naim, M., Advento, A. D. & Caliman, J. P. The effect of pesticide residue on mortality and fecundity of Elaeidobius kamerunicus (Coleoptera: Curculionidae). in *SOUTHEAST ASIA PLANT PROTECTION CONFERENCE 2019* (ed. Hidayat, SH and Damayanti and Adam, NA and Giyanto and Sartiami, D) vol. 468 (IPB Univ, Fac Agr, Dpet Plant Protect; Int Soc SE Asian Agr Sci Indonesia Chapter; Univ Putra Malaysia, 2020).

Setyawan et al. (2020) investigated how pesticide residues in palm oil plantations affect a pollinating weevil (*Elaeidobius kamerunicus*). Palm flowers were sprayed with 1 of 15 pesticides before weevils were introduced then mortality and fecundity were measured over a 10 hour period. Glyphosate (480g/L) and triclopyr (670g/L) were both applied at 0.3% and resulted in 33% and 63% mortality, respectively. They also found that weevil mortality was highly correlated with weevil fecundity.

EFFICACY

Patton, A. J., Weisenberger, D. V. & Liu, W. Efficacy of triclopyr and synthetic auxin herbicide mixtures for common blue violet (Viola sororia) control. *WEED TECHNOLOGY* 34, 475–481 (2020).

Patton et al. (2020) evaluated various auxin herbicides and their ability to control the weed, common blue violet (*Viola sororia*). They compared triclopyr and 2,4-D as well as mixtures containing one or both of these herbicides. They found that triclopyr was the most effective at controlling *V. sororia*. A minimum of 75% containment was achieved when triclopyr was applied at rates of 0.84 kg/ha and above.

1.5 *CLOPYRALID*

No papers were published in 2020 describing the human health effects, ecological risk, or environmental fate of clopyralid.

1.6 *CLETHODIM*

NON-TARGET IMPACTS

Breda-Alves, F. *et al.* Clethodim (herbicide) alters the growth and toxins content of Microcystis aeruginosa and Raphidiopsis raciborskii. *CHEMOSPHERE* 243, (2020).

Breda-Alves et al. (2020) investigated how clethodim exposure affects health and toxin production in two species of cyanobacteria (*Microcystis aeruginosa* and *Raphidiopsis raciborskii*). Both species were exposed to 1-400 mg/L of clethodim for 4 days; growth and toxin content were monitored. At low doses, cell density increased, but decreased at high doses. Cyanotoxins increased at 50 mg/L and above in *M. aeruginosa*, but not in *R. raciborskii*.

Siddiqui, S. & Al-Rumman, S. Clethodim induced pollen sterility and meiotic abnormalities in vegetable crop Pisum sativum L. *CARYOLOGIA* 73, 37–44 (2020).

Siddiqui & Al-Rumman (2020) investigated how clethodim exposure affects pollen viability and cell replication in *Pisum sativum* (pea crop). Seeds were exposed to 0.01, 0.02, 0.03, and 0.04% of clethodim for one hour, then planted and cared for until maturity. The percentage of sterile pollen increased for all concentrations and was as high as 83%. Only the highest concentration induced meiotic abnormalities.

EFFICACY

Bianchi, L. *et al.* Effects of glyphosate and clethodim alone and in mixture in sourgrass (Digitaria insularis). *CROP PROTECTION* 138, (2020).

Bianchi et al. (2020) evaluated the efficacy of glyphosate, clethodim, and glyphosate + clethodim use in controlling glyphosate-resistant and glyphosate-susceptible sourgrass (*Digitaria insularis*). Clethodim was more effective at low doses than glyphosate for both biotypes. Concentrations of 14 and 15.9 g/ha of clethodim achieved 90% control whereas 176.8 and 852.4 g/ha of glyphosate resulted in only 50% control for the susceptible and resistant biotypes, respectively. Applications of 135g/ha of glyphosate and 12 g/ha of clethodim in mixture led to 80% control.

Bowen, A. K. M., Beauchamp, V. B. & Stevens, M. H. H. Evaluating the efficacy of removal treatments on wavyleaf basketgrass (Oplismenus undulatifolius). *INVASIVE PLANT SCIENCE AND MANAGEMENT* 13, 176–188 (2020).

Bowen et al. (2020) evaluated different treatment methods for controlling *Oplismenus undulatifolius* (wavyleaf basketgrass) and identified which treatments had the least effect on native plant species. Of the eight tested treatments, glyphosate, hand weeding, and clethodim were found to be most effective. A one-time application of glyphosate and hand weeding resulted in reductions in *O. undulatifloius* cover by at least 91% whereas clethodim only achieved 48%. However, when clethodim was applied annually over a three-year period, it was equally if not more effective than the other treatments. Additionally, clethodim was the least harmful to native species richness.

Brunori, A. M. & Puricelli, E. C. Effect of herbicides and doses on short- and long-term control of Eleusine tristachya. *WEED RESEARCH* 60, 259–268 (2020).

Brunori & Puricelli (2020) found that glyphosate, haloxyfop-methyl, and clethodim were capable of controlling *Eleusine tristachya*. Glyphosate and clethodim provided greater than 90% control when applied at roughly 500 g/ha and 100 g/ha. However, greater doses were necessary when the plant was in its reproductive stage.

Metier, E. P., Lehnhoff, E. A., Mangold, J., Rinella, M. J. & Rew, L. J. Control of downy brome (Bromus tectorum) and Japanese brome (Bromus japonicus) using glyphosate and four graminicides: effects of herbicide rate, plant size, species, and accession. *WEED TECHNOLOGY* 34, 284–291 (2020).

Metier et al. (2020) studied the efficacy of herbicide use in controlling two species of invasive grasses *Bromus tectorum* and *Bromus japonicus*. They tested multiple rates of 5 herbicides, including glyphosate and clethodim, on plants of varying size. For newly germinated plants, less than 5 cm, all tested herbicides similarly reduced biomass. However, at larger sizes low doses of glyphosate (0.42 kg/ha) were ineffective, but low doses of clethodim (0.076 kg/ha) and other herbicides reduced biomass. The tallest plants (17 cm) were minimally affected by herbicide application. There was no difference in effectiveness between the low and high doses of the non-glyphosate herbicides (clethodim, sethoxydim, fluazifop, quizalofop).

2.0 *SURFACTANTS/ADJUVANTS*

No papers were published in 2020 describing the human health effects, ecological risk, or environmental fate of the adjuvants or surfactants used by MROSD.

3.0 *FUNGICIDES*

No papers were published in 2020 describing the human health effects, ecological risk, or environmental fate of fungicides used by MROSD.

4.0 *INSECTICIDES*

4.1 *DIATOMACEOUS EARTH*

No papers were published in 2020 describing the human health effects, ecological risk, or environmental fate of diatomaceous earth.

4.2 *D-TRANS ALLETHRIN*

No papers were published in 2020 describing the human health effects, ecological risk, or environmental fate of D-trans allethrin when used as an insecticide.

4.3 *FIPRONIL*

HUMAN HEALTH

Bae, J.-W. & Kwon, W.-S. Investigating the effects of fipronil on male fertility: Insight into the mechanism of capacitation. *REPRODUCTIVE TOXICOLOGY* 94, 1–7 (2020).

Bae & Kwon (2020) exposed mouse sperm to 0.1-300 µM of fipronil to assess whether or not it interferes with cellular signaling related to motility and function. At concentrations of 1 µM and above, various motility parameters were negatively affected, such as overall sperm motility and average path velocity. Capacitated sperm, sperm that are mature enough to fertilize, decreased at 300 µM. Additionally, intracellular ATP (a critical energy molecule) was reduced at high concentrations.

Beranger, R. *et al.* Multiple pesticides in mothers' hair samples and children's measurements at birth: Results from the French national birth cohort (ELFE). *INTERNATIONAL JOURNAL OF HYGIENE AND ENVIRONMENTAL HEALTH* 223, 22–33 (2020).

Beranger et al. (2020) used elastic net regression modeling to examine the association between maternal pesticide exposure and children's size at birth. They collected hair samples from over 300 French mothers shortly after birth and measured birth length, weight, and head circumference of the children. Fipronil and its metabolite fipronil sulfone, were two of the 64 pesticides detected in the hair samples; the detection frequency was 87% for fipronil and 99% for fipronil sulfone. Fipronil sulfone was one of the 8 pesticides selected as a predictor of birth weight. Birth weight was higher for children whose mother's hair contained moderate amounts of fipronil sulfone. These findings show a correlation between pesticide exposure during pregnancy and altered birth size in a relatively small cohort, but more research is needed to substantiate these findings.

4.4 *INDOXACARB*

No papers were published in 2020 describing the human health effects, ecological risk, or environmental fate of indoxacarb.

4.5 *PHENOTHRIN*

No papers were published in 2020 describing the human health effects, ecological risk, or environmental fate of phenothrin.

4.6 *PRALLETHRIN*

No papers were published in 2020 describing the human health effects, ecological risk, or environmental fate of prallethrin.

4.7 *S-HYDROPRENE*

No papers were published in 2020 describing the human health effects, ecological risk, or environmental fate of S-hyrdoprene.

4.8 *SODIUM TETRABORATE DECAHYDRATE*

No papers were published in 2020 describing the human health effects, ecological risk, or environmental fate of sodium tetraborate decahydrate.

5.0 RODENTICIDES

No papers were published in 2020 describing the human health effects, ecological risk, or environmental fate of rodenticides used by MROSD.