Midpeninsula Regional Open Space District Integrated Pest Management Program

2021-2023 Literature Review Technical Report

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Executive Summary

Midpeninsula Regional Open Space District (District) is a regional greenbelt system which manages over 63,000 acres of open space land and 26 open space preserves. As part of its land management approach, the District practices Integrated Pest Management (IPM) to safely and effectively control pests in a manner that minimizes potential risk to humans, wildlife, and the environment. To evaluate recent research on pesticides used by the District and their effect on the natural and human environments, a literature review of relevant peer-reviewed publications released between 2021 and 2023 was conducted and summarized in this technical report.

Several studies investigated the effects on pollinators and aquatic organisms (e.g., fish, aquatic invertebrates, and frogs) when exposed to pesticides at doses lower than those expected to cause mortality. While exposure to glyphosate generally did not impact survival in bumblebees, some studies reported observations of sublethal effects such as stress, behavioral effects, and shifts in the gut microbiome, particularly when other environmental stressors were also present. The potential for developmental, neurotoxic, and physiological effects resulting from glyphosate and fipronil exposure during the vulnerable life stages of aquatic organisms was also demonstrated. These effects were more pronounced in organisms exposed to formulated products as opposed to the active ingredient alone. Studies on soil microbes showed that impacts on microbial communities exposed to pesticides, if any, are often temporary and reversible after a period of acclimation.

The health and safety of glyphosate, triclopyr, and fipronil was evaluated in several studies, though the environmental relevance of the results was not always clear. For example, while maternal glyphosate exposure in rats was associated with the development of autism spectrum disorder-like behaviors, the authors acknowledged that similar exposures in humans were unlikely to occur. The potential for reproductive, developmental, and behavioral effects from glyphosate exposure is likely to be a continued area of study. Studies on triclopyr and fipronil suggest that these chemicals are not expected to cause reproductive or developmental toxicity in humans.

The efficacy and effectiveness of several herbicide, insecticide, and rodenticide active ingredients was validated in the studies reviewed. While important tools in IPM programs, overreliance on or improper use of pesticides can indirectly affect biodiversity.

Overall, the findings from the literature emphasize the importance of using pesticides judiciously and in a manner that minimizes risks to humans, wildlife, and the environment. The District's continued implementation of site monitoring, adherence to pesticide label instructions, and selective application of pesticides in combination with non-chemical tools is recommended to reduce the potential for offsite movement and non-target effects.

1. Introduction

Midpeninsula Regional Open Space District (District) is a regional greenbelt system which manages over 63,000 acres of open space land and 26 open space preserves. The District includes parts of San Mateo, Santa Clara, and Santa Cruz counties and provides protection for local wildlife habitats necessary to sustain plant and animal life and natural resources. As part of its land management approach, the District practices Integrated Pest Management (IPM) to safely and effectively control pests while minimizing risk of adverse impacts to non-target receptors.

The purpose of this technical report is to summarize and evaluate recent peer-reviewed research on pesticides and their effect on the natural and human environments. Based on the findings from academic research papers released during 2021-2023, recommendations for IPM Program activities are provided.

2. Literature Review

2.1. Approach

Google Scholar was used to conduct a comprehensive literature review of peer-reviewed research released between 2021 and 2023 which studied active ingredients currently approved for use under the District's IPM Program. Due to the sheer mass of publications released during this timeframe, the following criteria were used to select research articles for further review:

- Article focused on one or more of the following topics, as it relates to land management with pesticides in natural areas (including timber) or rangeland: biodiversity, water quality, soil health, wildlife, efficacy and effectiveness, human health and safety, and drift and effects on non-target species;
- Published in a peer-reviewed journal with an Impact Factor[1] of 4 or higher;
- Publication was readily accessible for review; and
- Included active ingredient name in the publication title.

Professional judgement on study quality and relevance was also used as appropriate. Papers meeting these criteria are included in the annotated bibliography provided in **Attachment 1**.

2.2. Summary of Findings

The two active ingredients most heavily researched during 2021-2023 and potentially relevant to Program activities included the herbicide glyphosate and the insecticide fipronil. Several publications on the rodenticide cholecalciferol and the insecticide diatomaceous earth were also identified but were of minimal relevance to the District's IPM Program. For example, studies on cholecalciferol (i.e., Vitamin D) were focused on its medicinal uses while studies on diatomaceous earth were focused on the control of stored grain insect pests. A summary of key findings from published literature is provided in the subsections below.

[1] The Impact Factor represents the average number of times articles in a journal have been cited in a particular year or period.

Note that no relevant publications released from 2021 to 2023 which met the criteria listed above were found for potassium phosphite salts, aminopyralid, clethodim, D-trans allethrin, prallethrin, S-hydroprene, sodium tetraborate decahydrate, or adjuvants used by the District.

2.2.1. Wildlife and Biodiversity

As it relates to wildlife effects, the primary focus areas of the published literature included impacts to pollinators and aquatic species. Studies in bumblebees found that while acute exposure to glyphosate did not significantly impact survival, sublethal effects such as stress, behavioral effects, and shifts in the gut microbiome which could later affect overall colony fitness may occur, particularly under study conditions representing bees which solely foraged on glyphosate-treated crops. Similarly, bumblebee exposure to sublethal doses of fipronil resulted in behavioral impacts such as increased walking speed and velocity, changes in midgut morphology, and increased antioxidant activity. Other sublethal effects reported following glyphosate exposure included impaired learning of aversive stimuli, impaired learning and memory of fine-color discrimination, and reduced the ability of bumblebee colonies to maintain optimal brood temperatures during periods of resource limitation. Foraging behavior, food consumption, olfaction, general vision, locomotion, photoaxis were not significantly impacted by glyphosate. In a study of high acute exposure of bumblebees, glyphosate did not cause significant impact on bee mortality, parasite intensity, or bacterial composition of the gut microbiome.

Toxicity studies in rainbow trout suggest that glyphosate exposure results in decreased bacterial diversity on gill microbiota but does not significantly impact survival. Conflicting information was found on whether glyphosate exposure leads to increased susceptibility to viral infections in juvenile trout, though this effect appears to be more pronounced in studies on glyphosate-based herbicide exposure as opposed to glyphosate alone. Increased swimming activity was observed after intergenerational exposure of rainbow trout larvae to glyphosate, while decreased locomotor activity was seen in zebrafish larvae acutely exposed to glyphosate. The potential for developmental toxicity was reported following glyphosate exposure in studies on rainbow trout, zebrafish, and frogs. Genotoxicity and impacts on aquatic invertebrates were typically more pronounced in studies involving formulated products containing glyphosate or the primary metabolite of glyphosate, aminomethylphosphonic acid (AMPA). In frog tadpoles exposed to an imazapyr-based product, developmental effects and reduced thyroid gland area were also reported, though further study is needed. Furthermore, insecticides such as fipronil are commonly understood to be toxic to aquatic organisms. Observations in support of this understanding were reported in the literature, emphasizing the importance of reducing or eliminating offsite movement of pesticides, and in particular insecticides, to aquatic habitats.

In a study conducted in banana plantations in Martinique, frequent glyphosate use led to lower species richness in predators, omnivores, herbivores, and decomposers in treated plots while native species became more prevalent in non-treated plots. These findings suggest that impacts to biodiversity may occur as an indirect result of reliance on pesticides for the purposes of land management in lieu of a more balanced, IPM-oriented approach which incorporates the use of non-chemical tools and the strategic use of pesticides.

2.2.2. Soil Health

Studies on soil microbes exposed to pesticides showed that impacts to microbial communities are often transient. Sun et al. (2022) found that low concentrations of clopyralid initially inhibited bio-denitrification activities and reduced richness and biodiversity in soil microbial communities, but recovery was noted after a period of acclimation.

Similarly, when glyphosate was applied in a forest restoration setting, soil bacterial communities temporarily shifted in treated plots compared to untreated plots before recovering. Glyphosate and AMPA did not cause significant changes in benthic sediment microbial community composition, diversity or richness in a prairie hole wetland microcosm study.

Exposure to fipronil also temporarily decreased the abundance and activity of soil microbes, though these effects were no longer observed 180 days after application. Similarly, in a field study, fipronil caused temporary inhibition of enzymatic activities, with recovery noted by the end of the 2-year study period. In a toxicity study on the effects of fipronil exposure in freshwater environments, the metabolic profiles of aquatic and soil bacteria were minimally affected.

2.2.3. Water Quality

Glyphosate was shown to break down quickly by photodegradation in aquatic settings. Due to nutrients released from aquatic plants affected by glyphosate, applications of glyphosate in such settings can encourage algal growth. When used to make forestry applications in Canada, a water quality monitoring program covering eight river systems detected glyphosate at low concentrations in only one of nearly 300 water samples collected, suggesting that judicious use of glyphosate according to label instructions is unlikely to impair water quality.

2.2.4. Human Health and Safety

The health and safety of glyphosate, triclopyr, and fipronil was evaluated in several studies, though the environmental relevance of the results was not always clear. The endocrine-disrupting, cytotoxic, genotoxic, developmental and reproductive toxicity, and neurotoxic potential of glyphosate in vitro, for example, has been hypothesized in both review articles and laboratory tests, though more research is needed to validate these findings and to determine whether exposures similar to those tested are likely to occur in practice. Studies and review articles on glyphosate consistently found that glyphosate-based herbicides were more toxic than glyphosate itself, indicating that co-formulants likely contribute to the impacts observed. For example, glyphosate-based herbicides were reported to cause necrosis in human cell lines, neurotoxicity, and alter gut microbiota more so than or instead of glyphosate alone. In a study on mouse embryonic stem cells exposed to glyphosate, no cytotoxic or genotoxic effects were observed.

Exposure to triclopyr did not show specific reproductive toxicity or fetotoxicity in rabbits or developmental/reproductive toxicity in rats. Similarly, no specific developmental neurotoxicity was observed in human cells exposed to fipronil. In studies evaluating the cognitive impacts of pesticide exposure, glyphosate showed potential in impairing recognition and spatial memory in rats. Autism spectrum disorder-like behaviors resulting from maternal glyphosate exposure were also reported in one study, though the authors note that exposures of similar magnitude were unlikely to happen in practice. Interestingly, fipronil was reported to affect emotional and cognitive behaviors in mice by increasing dopamine and serotonin levels in the brain.

2.2.5. Efficacy and Effectiveness

The efficacy and effectiveness of several active ingredients was validated in the studies reviewed. For vegetation management, glyphosate was shown to be an effective tool in forestry settings. When used for riparian forest restoration, glyphosate reduced weed biomass and invasive grass more effectively than mowing. Glyphosate was also found to be an important tool in natural pine control, where 100% burnout was achieved after one year of treatment. Imazapyr alone did not provide sufficient control, but it improved when mixed with glyphosate. In a study on glyphosate-resistant oat species in Australia, clopyralid was identified as an effective alternative.

For insect management, the efficacy of fipronil, indoxacarb, and phenothrin was validated. Fipronil was effective in controlling social insects such as the leaf-cutting ant due to its activity through both contact and oral exposure. Fipronil-containing grain bait and deer feed was tested in several studies, with preliminary results indicating its effective use in flea and tick control programs and disease mitigation in species such as black-tailed prairie dogs, white-footed mice, and white-tailed deer. The efficacy of indoxacarb was also demonstrated in a dose-dependent manner in stink bugs and the common armyworm. Because these species were not considered the target insects in the studies, the need for careful consideration of impacts to non-target species was strongly emphasized. For mosquito control, the use of propylene glycol and glycerol as adjuvants in phenothrin formulations was investigated. Both formulations, particularly the propylene glycol-added formulation, improved the efficacy of phenothrin on adult mosquitoes but had minimal efficacy against immature stages.

For rodent management, the effectiveness of cholecalciferol on wood rats was compared to that of chlorophacinone and warfarin. While cholecalciferol-containing baits had the lowest consumption levels, the effectiveness of cholecalciferol was comparable to that of chlorophacinone and much better than warfarin. In a secondary poisoning assessment, no effects were seen in barn owls for 6 months.

2.3. Potential for Research Author Bias and Conflicts of Interest

It is important to acknowledge the potential for research author bias and conflicts of interest when reviewing academic research. Caution is highly recommended when reading review articles and surveys. Review articles, for instance, reflect authors’ opinions on data evaluated in other studies as opposed to drawing their own conclusions based on a body of newly generated data. Epidemiology studies such as case control and cohort studies may introduce intentional or unintentional bias, for example, when questions are hard to understand or are asked in a manner which is more likely to result in a specific response. Additionally, bias of study participants must be considered. A common example of participant bias is recall bias, which occurs when participants incorrectly remember or report previous events or experiences.

Some bias also originates in the publishing process. The page charge associated with publishing in prestigious journals, for example, may influence authors of sound research to publish in lesser known or smaller journals with no page charge. Funding to complete and publish a given study tends to be more readily available for controversial issues and less readily available for studies that address more mundane topics.

The quality of a given study can be difficult to characterize and is often based on the scientific integrity of the researcher as opposed to the researcher's affiliation. While conflict of interest statements made in published research papers may be informative in some cases, generally speaking they are not always a useful indicator of study quality. Use of resources such as Retraction Watch can be helpful by providing information on research articles which have been retracted and the reason(s) for retraction (e.g., falsified or reused data, author bias, conflicts of interest, and errors).

3. Management Implications and IPM Program Recommendations

Overall, the findings from the literature emphasize the importance of using pesticides judiciously and in a manner that minimizes risks to humans, wildlife, and the environment. Uncertainty exists as to how inert ingredient(s) or co-formulant(s), if any, contribute to toxicity when the identity of these chemicals is often considered trade secret. The continued implementation of site monitoring, adherence to pesticide label instructions, and selective application of pesticides is recommended to reduce the potential for non-target effects. Special attention should be given to areas used as breeding sites and where non-target species biologically similar to the target pest may be present, as well as in areas where there is potential for offsite movement of pesticides.

Attachment 1

2021-2023 Annotated Bibliography

Midpeninsula Regional Open Space District
Integrated Pest Management Program

2021-2023
Annotated Bibliography

August 2024

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1. FUNGICIDES

1.1. Potassium Phosphite Salts

No relevant publications released from 2021 to 2023 which focused on one or more of the following topic areas were found for this chemical: biodiversity, water quality, soil health, wildlife, efficacy and effectiveness, human health and safety, and drift and effects on non-target species.

2. HERBICIDES

2.1. Aminopyralid

No relevant publications released from 2021 to 2023 which focused on one or more of the following topic areas were found for this chemical: biodiversity, water quality, soil health, wildlife, efficacy and effectiveness, human health and safety, and drift and effects on non-target species.

2.2. Clethodim

No relevant publications released from 2021 to 2023 which focused on one or more of the following topic areas were found for this chemical: biodiversity, water quality, soil health, wildlife, efficacy and effectiveness, human health and safety, and drift and effects on non-target species.

2.3. Clopyralid

Sun, S., Hou, Y.-N., Wei, W., Sharif, H. M. A., Huang, C., Ni, B.-J., Li, H., Song, Y., Lu, C., Han, Y., & Guo, J. (2022). Perturbation of clopyralid on bio-denitrification and nitrite accumulation: Long-term performance and biological mechanism. *Environmental Science and Ecotechnology, 9*, 100144.

Sun et al. (2022) investigates the long-term effects of clopyralid on nitrite accumulation and microbial community responses during the bio-denitrification process. While low concentrations of clopyralid (<15 mg/L) initially resulted in nitrite accumulation, higher concentrations had no further impact after an acclimation period of roughly 80 days. After acclimating, metabolic activities that had been inhibited by clopyralid were restored and a U-shaped recovery of biodiversity and richness in the microbial community was observed, indicating that the microbial communities adapted over time.

2.4. Glyphosate

An, J., Jiang, Y., Cao, H., Yi, C., Li, S., Qu, M., & Liu, G. (2023). Photodegradation of glyphosate in water and stimulation of by-products on algae growth. *Ecotoxicology and Environmental Safety, 263*, 115211.

An et al. (2023) studies the photodegradation of glyphosate in various water bodies (ditches, ponds, and lakes) and the impact of its degradation products on algae growth. The results indicate that aqueous photodegradation is a significant degradation pathway for glyphosate, showing a degradation rate of up to 86% in ditches after 96 hours. The authors also report that the phosphate released from glyphosate degradation can promote growth of the cyanobacteria *Microcystis aeruginosa*, a species which can form harmful algal blooms in eutrophic lakes.

Anunciato, V. M., Bianchi, L., Gomes, G. L. G. C., Velini, E. D., Duke, S. O., & Carbonari, C. A. (2021). Effect of low glyphosate doses on flowering and seed germination of glyphosate-resistant and -susceptible *Digitaria insularis*. *Pest Management Science, 78*(3), 1227-1239.

Anunciato et al. (2021) examines the hormetic effects of low glyphosate doses on glyphosate-resistant and -susceptible biotypes of sourgrass (*Digitaria insularis)*. The study reports that low doses of glyphosate stimulated early flowering, increased seed weight, and enhanced germination speed in both biotypes. The glyphosate-resistant biotype required higher doses to achieve these effects than the susceptible biotype, suggesting that the hermetic effects could benefit the plant and potentially facilitate its spread in glyphosate-treated areas.

Bellec, L., Le Du-Carrée, J., Almeras, F., Durand, L., Cambon-Bonavita, M.-A., Danion, M., & Morin, T. (2022). Glyphosate-based herbicide exposure: effects on gill microbiota of rainbow trout (*Oncorhynchus mykiss*) and the aquatic bacterial ecosystem. *FEMS Microbiology Ecology, 98*(8), fiac076.

This study examines the impact of chronic exposure to glyphosate and glyphosate-based herbicides on the gill microbiota of rainbow trout (*Oncorhynchus mykiss*). Results revealed a significant decrease in bacterial diversity in the gills of exposed fish. The authors note that the consequences of these findings remain unknown and require further studies at the functional level.

Chauhan, B. S. (2022). The world's first glyphosate-resistant case of *Avena fatua* L. and *Avena sterilis* ssp. *ludoviciana* (Durieu) Gillet & Magne and alternative herbicide options for their control. *PLOS ONE, 17*(1), e0262494.

This study identifies glyphosate-resistant biotypes of common wild oat (*Avena fatua*) and winter wild oat (*Avena sterilis* ssp. *Ludoviciana*) in Australia. The glyphosate dose required to kill 50% of the resistant biotypes (LD50) was 556 g a.e./ha (0.49 lb/ac) for *A. fatua* and 848 g a.e./ha (0.75 lb/ac) for *A. ludoviciana*. Clethodim was noted as an effective alternative herbicide, regardless of growth stage.

Cornish, C. M., Bergholz, P., Schmidt, K., & Sweetman, J. (2023). How benthic sediment microbial communities respond to glyphosate and its metabolite: A microcosm experiment. *Microbial Ecology, 86*(3), 2949-2958.

This study investigates the effects of glyphosate and its metabolite aminomethylphosphonic acid (AMPA) on benthic sediment microbial communities in a prairie pothole wetland. Using 16S rRNA gene sequencing, the authors analyzed microbial community composition after exposure to different concentrations of glyphosate and AMPA. Results showed no significant changes in microbial community composition, diversity, or operational taxonomic unit (OTU) counts across treatments and concentrations, indicating tolerance to glyphosate.

Cullen, M. G., Bliss, L., Stanley, D. A., & Carolan, J. C. (2023). Investigating the effects of glyphosate on the bumblebee proteome and microbiota. *Science of the Total Environment*, *864,* 161074.

Cullen et al. (2023) examines the impact of glyphosate on the proteome and microbiota of the bumblebee *Bombus terrestris*. Results showed no significant differences in bee survival, behavior, or sucrose solution consumption between treatment and control groups. However, proteomic analysis revealed changes in proteins related to oxidative stress regulation, metabolism, cellular adhesion, and extracellular matrix. The authors conclude that sublethal impacts of glyphosate exposure as well as the impacts of co-formulants should be further studied.

Darmani, H., & Al-Saleh, D. R. H. (2023). Lower concentrations of the glyphosate-based herbicide Roundup® cause developmental defects in *Artemia salina*. *Environmental Toxicology and Chemistry, 42(7)*, 1586-1594.

This study evaluates the impact of various concentrations of glyphosate in Roundup Weed & Grass Killer on brine shrimp. Mortality was observed at 288 µg/ml, whereas lower concentrations did not significantly affect viability. At 0.72 µg/ml, reduced body length and widths of the tail, abdomen, and head were observed. Based on these findings, the authors conclude that environmentally relevant concentrations of glyphosate in the formulated product Roundup Weed & Grass Killer can adversely impact development in non-target aquatic invertebrate species.

Edge, C. B., Haines, W., Blaney, M., & Noël, M. (2023). Low detection of glyphosate in rivers following application in forestry. *Pest Management Science*, 79, 2951-2958.

This study assessed the frequency and concentration of glyphosate and AMPA in surface waters following forestry applications in New Brunswick, Canada. Across three monitoring programs, 296 water samples from eight river systems were analyzed over two years. Glyphosate was detected in only one sample at 17 ppb, and AMPA was not detected in any samples. The findings suggest that glyphosate is unlikely to be present in surface waters during baseflow conditions due to infrequent applications and the high capacity of soil to bind glyphosate.

El Jaouhari, M., Damour, G., Tixier, P., & Coulis, M. (2023). Glyphosate reduces the biodiversity of soil macrofauna and benefits exotic over native species in a tropical agroecosystem. *Basic and Applied Ecology, 73,* 18-26.

In this study, the authors evaluated the impact of glyphosate application on soil macrofauna in banana plantations in Martinique. The results showed that macrofauna species richness decreased by 21% in plots with the highest frequency of herbicide application. Specifically, species richness decreased by 22% for predators, 17% for omnivores, 55% for herbivores, and 55% for decomposers in heavily treated plots compared to those with no herbicide use. The study also noted a significant shift in species composition, with native species being more prevalent in herbicide-free plots, suggesting that reducing herbicide use could enhance soil biodiversity conservation.

Faria, M., Bedrossiantz, J., Rosas Ramírez, J. R., Mayol, M., Heredia García, G., Bellot, M., Prats, E., Garcia-Reyero, N., Gómez-Canela, C., Gómez-Oliván, L. M., & Raldúa, D. (2021). Glyphosate targets fish monoaminergic systems leading to oxidative stress and anxiety. *Environment International, 146*, 106253.

Exposure to environmentally relevant concentrations of glyphosate (0.3 and 3 µg/L) for two weeks impaired exploratory and social behaviors in adult zebrafish, indicating increased anxiety. Neurochemical analysis revealed a significant increase in dopamine and serotonin levels in the anterior brain, alongside increased catalase and superoxide dismutase activities, and decreased glutathione stores, leading to oxidative stress.

Flach, H., Dietmann, P., Liess, M., Kühl, M., & Kühl, S. J. (2023). Glyphosate without Co-formulants affects embryonic development of the South African clawed frog *Xenopus laevis*. *Ecotoxicology and Environmental Safety, 260*, 115080.

This study investigated the impact of glyphosate on the embryonic development of *Xenopus laevis*. Results showed that acute glyphosate exposure led to significant morphological changes including reduced body length, head area, and eye size at the 0.1 mg/L dose level. Cardiac development was adversely affected, with significant reductions in heart size and heart rate observed at the same concentration. The authors conclude that glyphosate impairs the embryogenesis of *X. laevis*, potentially impacting amphibian populations in natural habitats as a result of impaired development and increased vulnerability to environmental stressors.

Florido, F. G., Regitano, J. B., Andrade, P. A. M., Andreote, F. D., & Brancalion, P. H. S. (2022). A comprehensive experimental assessment of glyphosate ecological impacts in riparian forest restoration. *Ecological Applications, 32*(1), e02472.

In this study, glyphosate spraying in riparian forest restoration reduced weed biomass by half and invasive grass *Urochloa decumbens* by one-third compared to mowing treatments. Planted tree seedlings showed significant improvements in height, crown area, and basal area, and the regeneration of native woody and ruderal herbaceous plants was enhanced. Despite no detection of glyphosate or AMPA residues in soil and water, high concentrations were found in runoff sediments. Soil bacterial communities shifted in glyphosate-treated plots compared to mowing plots, though this effect was transient.

Forner-Piquer, I., Faucherre, A., Byram, J., Blaquiere, M., de Bock, F., Gamet-Payrastre, L., Ellero-Simatos, S., Audinat, E., Jopling, C., & Marchi, N. (2021). Differential impact of dose-range glyphosate on locomotor behavior, neuronal activity, glio-cerebrovascular structures, and transcript regulations in zebrafish larvae. *Chemosphere, 267*, 128986.

The study investigated the effects of various glyphosate concentrations on zebrafish larvae, focusing on behavior, neurophysiology, and cellular structures. High concentrations (≥1,000 mg/L) significantly decreased locomotor activity and altered spike activity in the midbrain, but no gross morphological changes were observed. Microglial activation observed when zebrafish larvae were exposed to both low (0.1 mg/L) and high (1,000 mg/L) concentrations of glyphosate levels from 1.5 to 120 hours post-fertilization, suggesting potential neuroinflammation as a result of exposure.

Fuchs, B., Saikkonen, K., & Helander, M. (2021). Glyphosate-modulated biosynthesis driving plant defense and species interactions. *Trends in Plant Science, 26*(4), 312-321.

This opinion article discusses how sublethal doses of glyphosate residues in soil impact may plant defense mechanisms and species interactions. Glyphosate disrupts the shikimate pathway, altering the production of amino acids which are used to produce plant defense compounds such as phenylpropanoids. This disruption is reported to lead to decreased production of compounds such as lignins, anthocyanins, and flavonoids and increased plant susceptibility to herbivores and pathogens. The authors suggest that these glyphosate-modulated changes reduce the attractiveness of plants to pollinators and other beneficial insects.

Gao, X., Hu, F., Zhang, S., Luo, J., Zhu, X., Wang, L., Zhang, K., Li, D., Ji, J., Niu, L., & Cui, J. (2021). Glyphosate exposure disturbs the bacterial endosymbiont community and reduces body weight of the predatory ladybird beetle *Harmonia axyridis* (Coleoptera: Coccinellidae). *Science of the Total Environment, 790*, 147847.

The study investigates the impact of glyphosate on predatory Asian lady beetle larvae, *Harmonia axyridis*. Results indicate that while glyphosate exposure did not significantly affect the survival rate, developmental duration, or pupation and emergence rates of *H. axyridis*, it did result in reduced the body weight of the beetles by 15-20% when exposed at the larval stage. The microbial community analysis revealed that glyphosate exposure significantly altered the relative abundance of the bacterial endosymbiont community, increasing the abundance of *Enterobacter* while decreasing *Staphylococcus*. These findings suggest that glyphosate's impact on microbial communities may indirectly affect the physiological health of non-target species such as *H. axyridis*, emphasizing the need for careful consideration of herbicide impacts beyond target weeds.

Helander, M., Jeevannavar, A., Kaakinen, K., Mathew, S. A., Saikkonen, K., Fuchs, B., Puigbò, P., Loukola, O. J., & Tamminen, M. (2023). Glyphosate and a glyphosate-based herbicide affect bumblebee gut microbiota. *FEMS Microbiology Ecology, 99*(1), 1-10.

This study investigates the impact of glyphosate and the glyphosate-based herbicide Roundup Gold on the gut microbiota of bumblebees (*Bombus terrestris*). Results show that glyphosate alone increased gut microbiota diversity, while Roundup Gold decreased it, indicating negative effects are primarily due to co-formulants. Both treatments significantly reduced the relative abundance of potentially glyphosate-sensitive *Snodgrasella alvi* but increased *Candidatus schmidhempelia* in glyphosate-treated bees. Overall, 50% of bacterial genera were potentially resistant to glyphosate, while 36% were sensitive. Note that Roundup Gold is not registered for use in California.

Helander, M., Lehtonen, T. K., Saikkonen, K., Despains, L., Nyckees, D., Antinoja, A., Solvi, C., & Loukola, O. J. (2023). Field-realistic acute exposure to glyphosate-based herbicide impairs fine-color discrimination in bumblebees. *Science of the Total Environment, 857*, 159298.

The study investigates the effects of the glyphosate-based herbicide Roundup Gold on bumblebee (*Bombus terrestris*) cognition. Acute exposure to a field-realistic dose of Roundup Gold impaired learning and memory of fine-color discrimination in bumblebees, while the control group showed improved performance. Olfaction, general vision, and foraging behavior did not appear to be affected. Note that Roundup Gold is not registered for use in California.

Herek, J. S., Vargas, L., Trindade, S. A. R., Rutkoski, C. F., Macagnan, N., Hartmann, P. A., & Hartmann, M. T. (2021). Genotoxic effects of glyphosate on *Physalaemus* tadpoles. *Environmental Toxicology and Pharmacology, 81, 103516*.

The study examines the genotoxic effects of the glyphosate-based product Original Roundup DI a glyphosate-based herbicide on the tadpoles of two South American amphibian species, *Physalaemus cuvieri* and *Physalaemus gracilis*. At all tested concentrations, there was evidence of cell damage, suggesting genotoxicity. The authors acknowledge that 75% of the Original Roundup DI formulation is proprietary and that co-formulants such as surfactants may have contributed to results. Note that Original Roundup DI is not registered for use in California.

Lacroix, R., & Kurrasch, D. M. (2023). Glyphosate toxicity: In vivo, in vitro, and epidemiological evidence. *Toxicological Sciences, 192*(2), 131-140.

In this review article, Lacroix and Kurrasch (2023) summarize in vivo, in vitro, and epidemiological studies on glyphosate toxicity. In vitro studies have observed that glyphosate and its formulations can induce cytotoxicity, genotoxicity, and endocrine disruption in human cell lines at certain concentrations, although relevance to environmental exposure levels is unclear. In vivo experiments suggest that glyphosate exposure could lead to significant physiological changes and transgenerational health effects in animal models, raising concerns about its safety. Epidemiological data indicates rising glyphosate levels in human biofluids, though evidence for direct health impacts is still inconclusive.

Le Du-Carrée, J., Boukhari, R., Cachot, J., Cabon, J., Louboutin, L., Morin, T., & Danion, M. (2021). Generational effects of chronic exposure to a low environmentally relevant concentration of glyphosate on rainbow trout, *Oncorhynchus mykiss*. *Science of the Total Environment, 801*, 149462.

This study investigates the generational toxicity of glyphosate on rainbow trout, focusing on developmental and physiological changes. Exposure to glyphosate resulted in increased head size and reduced cytochrome-c-oxidase activity in F2 generation fish. Additionally, there were behavioral changes such as reduced response to light in larvae exposed to glyphosate. Intergenerational exposure to glyphosate significantly increased the fish's susceptibility to hematopoietic necrosis virus, suggesting weakened immune responses.

Le Du-Carrée, J., Cabon, J., Morin, T., & Danion, M. (2021). Immunological and metabolic effects of acute sublethal exposure to glyphosate or glyphosate-based herbicides on juvenile rainbow trout, *Oncorhynchus mykiss*. *Science of the Total Environment, 784*, 147162.

This study evaluated the effects of acute sublethal exposure to glyphosate on juvenile rainbow trout, focusing on metabolic and immunological responses. After 96 hours of exposure to glyphosate, there were no significant changes in red and white blood cell counts for the glyphosate condition. Post-viral infection, cytochrome-c oxidase activity increased by 61%. Despite these physiological changes, glyphosate exposure did not significantly affect the overall survival rate post-viral infection, suggesting that glyphosate alone does not severely impair the fish's ability to handle viral challenges.

Le Du-Carrée, J., Morin, T., & Danion, M. (2021). Impact of chronic exposure of rainbow trout, Oncorhynchus mykiss, to low doses of glyphosate or glyphosate-based herbicides. *Aquatic Toxicology, 230*, 105687.

The study investigates the chronic effects of low-dose glyphosate and glyphosate-based herbicides (Roundup Innovert and Viaglif Jardin) on the health of rainbow trout (*Oncorhynchus mykiss*) over a 10-month period. Results showed no significant changes in reproductive parameters, oxidative stress markers, or metabolic activities for both glyphosate and the formulated products. While overall health was not significantly affected, a reduction in macrophage proportion and phagocytic activity observed in glyphosate-based herbicide-exposed trout two months before spawning suggested potential immunological effects. Note that neither Roundup Innovert nor Viaglif Jardin are registered for use in California.

Le Du-Carrée, J., Saliou, F., Cachot, J., Morin, T., & Danion, M. (2021). Developmental effect of parental or direct chronic exposure to environmental concentration of glyphosate on the larvae of rainbow trout, *Oncorhynchus mykiss*. *Aquatic Toxicology, 237, 105894*.

This study examines the effects of parental and direct exposure to 1 µg/L glyphosate and two glyphosate-based herbicides on the early developmental stages of rainbow trout (*Oncorhynchus mykiss*). While glyphosate exposure did not affect survival or induce malformations in larvae, direct exposure led to a 10% reduction in head size and intergenerational exposure significantly increased larval swimming activity.

Liang, D., Li, Y., Li, S., Meng, D., Li, F., Huang, S., Gong, M., Qin, J., & Li, H. (2023). Dose-dependent inhibitory effects of glyphosate on invasive *Pomacea canaliculata* reproductive and developmental growth under oxidative deposition. *Ecotoxicology and Environmental Safety*, 253, 114659.

This study investigates the potential reproductive and developmental impacts of glyphosate exposure on the channeled apple snail (*Pomacea canaliculate*). Glyphosate reduced the hatching rate and growth indices of *P. canaliculate* in a dose-dependent manner. Prolonged exposure resulted in observations of ovarian tissue damage and decreased fecundity. The study concludes that *P. canaliculata* can tolerate low concentrations of pollutants, but higher concentrations and prolonged exposure may adversely affect their reproduction and development.

Liu, J.-B., Li, Z.-F., Lu, L., Wang, Z.-Y., & Wang, L. (2022). Glyphosate damages blood-testis barrier via NOX1-triggered oxidative stress in rats: Long-term exposure as a potential risk for male reproductive health. *Environment International, 159*, 107038.

Liu et al. (2022) explored the impacts of glyphosate on the blood-testis barrier and sperm quality in rats exposed to glyphosate for 4 months via diet. Long-term exposure to glyphosate led to a significant decrease in sperm quality and quantity, disruption of blood-testis barrier integrity, and increased oxidative stress in the testis.

Liu, Z., Shangguan, Y., Zhu, P., Sultan, Y., Feng, Y., Li, X., & Ma, J. (2022). Developmental toxicity of glyphosate on embryo-larval zebrafish (*Danio rerio*). *Ecotoxicology and Environmental Safety, 236*, 113493.

Liu et al. (2022) investigated the developmental toxicity of glyphosate in zebrafish embryos exposed to various concentrations for 120 hours post-fertilization. Glyphosate exposure was associated with developmental abnormalities, including premature hatching, reduced heart rate, pericardial and yolk sac edema, swim bladder deficiency, and shortened body length.

Lopes, A., Benvindo-Souza, M., Carvalho, W. F., Nunes, H. F., de Lima, P. N., Costa, M. S., Benetti, E. J., Guerra, V., Saboia-Morais, S. M. T., Santos, C. E., Simões, K., Bastos, R. P., & de Melo e Silva, D. (2021). Evaluation of the genotoxic, mutagenic, and histopathological hepatic effects of polyoxyethylene amine (POEA) and glyphosate on *Dendropsophus minutus* tadpoles. *Environmental Pollution, 289*, 117911.

Lopes et al. (2021) studied the effects of glyphosate and the adjuvant polyoxyethylene amine (POEA) on lesser tree frog tadpoles (*Dendropsophus minutus*). Tadpoles exposed to POEA exhibited a 174% increase in DNA damage and a sevenfold rise in micronuclei at 5 µg/L, with all tadpoles dying at 10 µg/L. In comparison, glyphosate exposure resulted in 165% more DNA damage and a sixfold increase in micronuclei at 520 µg/L. Histopathological analysis revealed significant liver lesions in all exposure groups. While the results indicate that both compounds have genotoxic, mutagenic, and hepatotoxic effects, effects related to POEA exposure were apparent at much lower concentrations than those related to glyphosate exposure.

Luna, S., Neila, L. P., Vena, R., Borgatello, C., & Rosso, S. B. (2021). Glyphosate exposure induces synaptic impairment in hippocampal neurons and cognitive deficits in developing rats. *Archives of Toxicology, 95*(2137-2150).

In this study, Luna et al. (2021) evaluates the impact of glyphosate exposure on synaptic development and cognitive function in rats. When cultured hippocampal neurons were exposed to glyphosate for 5-10 days, reduced dendritic complexity and synaptic spine formation and maturation in hippocampal neurons was observed. Glyphosate injections in rat pups once every 48 hours for 3 weeks resulted in impaired recognition and spatial memory and correlated with reduced synaptic protein expression.

Maddalon, A., Galbiati, V., Colosio, C., Mandić-Rajčević, S., & Corsini, E. (2021). Toxicity of glyphosate and glyphosate-based herbicides. *Toxicology, 459*, 152851.

This review article discusses the impacts of glyphosate and various glyphosate-based herbicides on human health, wildlife, and microbiota. The authors found that glyphosate-based herbicides can significantly alter gut microbiota, promoting the growth of pathogenic bacteria over beneficial strains which could potentially lead to immune and inflammatory responses. The study also notes the higher toxicity of glyphosate-based herbicides compared to glyphosate alone, primarily due to the presence of adjuvants like POEA, which enhance their bioavailability and toxic effects.

Mesnage, R., Brandsma, I., Moelijker, N., Zhang, G., & Antoniou, M. N. (2021). Genotoxicity evaluation of 2,4-D, dicamba and glyphosate alone or in combination with cell reporter assays for DNA damage, oxidative stress and unfolded protein response. *Food and Chemical Toxicology, 157*, 112601.

This study investigates the cytotoxic and genotoxic effects of various herbicides individually and in combination on mouse embryonic stem cells. Glyphosate did not elicit a significant response.

Mesnage, R., Ferguson, S., Brandsma, I., Moelijker, N., Zhang, G., Mazzacuva, F., Caldwell, A., Halket, J., & Antoniou, M. N. (2022). The surfactant co-formulant POEA in the glyphosate-based herbicide RangerPro but not glyphosate alone causes necrosis in Caco-2 and HepG2 human cell lines and ER stress in the ToxTracker assay. *Food and Chemical Toxicology, 168*, 113380.

This study investigated the cytotoxicity of RangerPro, a glyphosate-based herbicide, compared to glyphosate and its surfactant co-formulant POEA in human Caco-2 and HepG2 cell lines. Results showed that RangerPro and POEA, but not glyphosate alone, induced significant cell necrosis in both cell lines. Additionally, only RangerPro and POEA caused endoplasmic reticulum stress, while glyphosate alone was associated with oxidative stress in HepG2 cells.

Meftaul, I. M., Venkateswarlu, K., Annamalai, P., Parven, A., & Megharaj, M. (2021). Glyphosate use in urban landscape soils: Fate, distribution, and potential human and environmental health risks. *Journal of Environmental Management, 292*, 112786.

This study evaluates the fate and distribution of glyphosate in urban soils and assesses the associated human and environmental health risks. The authors found that glyphosate sorption in soils is predominantly influenced by soil organic matter and clay content, with sorption kinetics best described by the pseudo-second-order model. Results suggest a high leaching potential of glyphosate, with leachability indices ranging from 0.31 to 1.0 and groundwater ubiquity scores between 1.60 and 3.44, suggesting significant risk of water contamination. Despite these findings, the calculated cancer risk and hazard quotient values indicate negligible carcinogenic and non-carcinogenic risks to humans from soil exposure.

Miranda, C. E., Clauser, C. D., Lozano, V. L., Cataldo, D. H., & Pizarro, H. N. (2021). An invasive mussel is in trouble: How do glyphosate, 2,4-D and its mixture affect *Limnoperna fortunei*'s survival? *Aquatic Toxicology, 239*, 105957.

This study investigates the impact of glyphosate, 2,4-D, and their mixture on the survival of the invasive mussel *Limnoperna fortunei*. Glyphosate, alone or combined with 2,4-D, significantly reduced mussel survival, particularly under conditions without air supply. The presence of herbicides disrupted dissolved oxygen dynamics, with glyphosate causing more pronounced dissolved oxygen fluctuations, contributing to mussel hypoxia and mortality.

Muñoz, J. P., Bleak, T. C., & Calaf, G. M. (2021). Glyphosate and the key characteristics of an endocrine disruptor: A review. *Chemosphere, 270, 128619*.

This review evaluates glyphosate's potential as an endocrine disruptor. The study finds that glyphosate meets at least eight of the ten key characteristics of endocrine-disrupting chemicals; however, further prospective cohort studies are necessary to fully elucidate its effects on the human endocrine system.

Nouvian, M., Foster, J. J., & Weidenmüller, A. (2023). Impact of long-term exposure to glyphosate on locomotion, phototaxis, and learning abilities in bumblebees. *Science of the Total Environment, 898*, 165527.

The study examined the effects of chronic exposure to sublethal concentrations of glyphosate on bumblebees. While glyphosate had a minimal and short-lived impact on locomotion and no effect on phototaxis, it significantly impaired learning of aversive stimuli when bumblebees were fed a glyphosate-sugar water solution for 30 to 64 days. These results suggest that long-term glyphosate exposure can disrupt cognitive processes critical for foraging and predator avoidance.

Odezulu, N. G., Lowney, Y. W., Nouri, M. Z., Roberts, S. M., & Stuchal, L. D. (2022). Assessment of the bioaccessibility of glyphosate in soil using a physiologically based extraction test. *Human and Ecological Risk Assessment: An International Journal, 28*(3-4), 316-325.

The study evaluated the bioaccessibility of glyphosate in different soil compositions using the Physiologically Based Extraction Test. Results showed that while soil-bound glyphosate residues are not readily bioaccessible, glyphosate bioavailability significantly increased under simulated gastrointestinal tract conditions. These findings may help inform exposure assumptions for human health risk assessment purposes.

Ojiro, R., Okano, H., Takahashi, Y., Takashima, K., Tang, Q., Ozawa, S., Zou, X., Woo, G.-H., & Shibutani, M. (2023). Comparison of the effect of glyphosate and glyphosate-based herbicide on hippocampal neurogenesis after developmental exposure in rats. *Toxicology, 483*, 153369.

This study examined the effects of glyphosate and glyphosate-based herbicide Turnout Liquid Agent on hippocampal neurogenesis in rats following developmental exposure. Results showed that both glyphosate and Turnout Liquid Agent induced limited impairments in hippocampal neurogenesis, mild oxidative stress responses, and altered synaptic plasticity. However, immunoreactive cellular responses and apoptotic cell induction were more pronounced with the formulated product, suggesting differential neurotoxic potential between glyphosate and Turnout Liquid Agent, likely influenced by surfactant presence. Note that Turnout Liquid Agent is not registered for use in California.

Pu, Y., Ma, L., Shan, J., Wan, X., Hammock, B. D., & Hashimoto, K. (2021). Autism-like behaviors in male juvenile offspring after maternal glyphosate exposure. *Clinical Psychopharmacology and Neuroscience, 19*(3), 554-558.

This study investigates the effects of maternal mouse exposure to high levels of glyphosate on autism spectrum disorder (ASD)-like behaviors in male juvenile offspring. Results showed significant increases in grooming behavior and social interaction deficits in glyphosate-exposed offspring compared to controls. Maternal exposure to glyphosate may cause ASD-like behaviors in juvenile offspring, although it is noted to be exceptionally unlikely that such exposure would occur during human pregnancy

Qu, M., Wang, L., Xu, Q., An, J., Mei, Y., & Liu, G. (2022). Influence of glyphosate and its metabolite aminomethylphosphonic acid on aquatic plants in different ecological niches. *Ecotoxicology and Environmental Safety, 246*, 114155.

This study examines the effects of glyphosate and AMPA on sweet flag (*Acorus calamus*), algae (*Chlorella vulgaris*), and tape grass (*Vallisneria natans*). Medium and low concentrations of glyphosate (≤0.5 mg/L) significantly inhibited the growth of *V. natans* and promoted the growth of *C. vulgaris* but had no significant effect on *A. calamus.* Combined exposure to glyphosate and AMPA showed additive or synergistic inhibition in *V. natans* at higher concentrations.

Ramula, S., Kalske, A., Saikkonen, K., & Helander, M. (2022). Glyphosate residues in soil can modify plant resistance to herbivores through changes in leaf quality. *Plant Biology, 24*(6), 979-986.

This study investigates the effects of glyphosate residues in soil on the plant resistance traits and herbivory of bigleaf lupine (*Lupinus polyphyllus*). Glyphosate treatment reduced leaf trichome density but did not affect other plant traits such as leaf mass per area, aboveground biomass, root ratio, nodule number, or nodule activity. Herbivore damage was not significantly influenced by glyphosate treatment, phosphorus fertilization, or plant height but increased over time. Glyphosate-exposed plants also had lower leaf consumption rates by land snails compared to control plants.

Silver, M. K., Fernandez, J., Tang, J., McDade, A., Sabino, J., Rosario, Z., Vélez Vega, C., Alshawabkeh, A., Cordero, J. F., & Meeker, J. D. (2021). Prenatal exposure to glyphosate and its environmental degradate, aminomethylphosphonic acid (AMPA), and preterm birth: A nested case–control study in the PROTECT cohort (Puerto Rico). *Environmental Health Perspectives, 129*(5).

Silver et al. (2021) studies the relationship between prenatal exposure to glyphosate and its degradant AMPA with preterm birth in the Puerto Rico Testsite for Exploring Contamination Threats (PROTECT) cohort in Puerto Rico. Results show that urinary glyphosate and AMPA levels at 26 weeks of gestation were associated with increased odds of preterm birth. Similar studies should be explored in other populations, preferably with larger sample sizes.

Sørensen, M. T., Poulsen, H. D., Katholm, C. L., & Højberg, O. (2021). Review: Feed residues of glyphosate – potential consequences for livestock health and productivity. *Animal, 15*, 100026.

This review explores the impact of dietary glyphosate residues on livestock health, focusing on gut microbiota and mineral status. While glyphosate has documented antimicrobial and mineral-chelating properties, in vivo evidence specific to livestock remains scarce. The antimicrobial effects of glyphosate may disrupt gut microbiota, favoring pathogenic bacteria over beneficial commensals. In cattle, glyphosate has been reported to affect fiber-degrading bacteria and overall microbiota composition. These findings indicate that glyphosate residues in livestock feed may have potential implications for animal productivity and health.

Strandberg, B., Sørensen, P. B., Bruus, M., & Damgaard, C. (2021). Effects of glyphosate spray-drift on plant flowering in non-target species. *Environmental Pollution, 280,* 116953.

This study investigates the impact of glyphosate-based herbicide Roundup Bio spray drift on the flowering of non-target plant species in an agricultural setting. The research showed significant reductions in the cumulative number of flowers for *Trifolium pratense* and *Lotus corniculatus* due to glyphosate exposure, while *Trifolium repens* exhibited no significant effects, and *Cichorium intybus* showed a non-significant positive effect. Glyphosate did not significantly affect the timing of flowering for any of the species studied. Note that Roundup Bio is not registered for use in California.

Straw, E. A., Mesnage, R., Brown, M. J. F., & Antoniou, M. N. (2023). No impacts of glyphosate or *Crithidia bombi*, or their combination, on the bumblebee microbiome. *Scientific Reports, 13*, 8949.

This study tested the effects of a high acute oral dose of glyphosate and its interaction with the gut parasite *Crithidia bombi* on the gut microbiome of the buff-tailed bumblebee (*Bombus terrestris*). The results indicated no significant impacts on bee mortality, parasite intensity, or the bacterial composition of the gut microbiome, with alpha and beta diversity showing no differences among treatments. The findings contrast with previous studies on honeybees, suggesting species-specific responses.

Strilbyska, O. M., Tsiumpala, S. A., Kozachyshyn, I. I., Strutynska, T., Burdyliuk, N., Lushchak, V. I., & Lushchak, O. (2022). The effects of low-toxic herbicide Roundup and glyphosate on mitochondria. *EXCLI Journal, 21*(183-196).

This review investigates the impact of glyphosate-based herbicides and glyphosate on mitochondrial functions in animals and humans. The study found that exposure to glyphosate and glyphosate-based herbicides may affect the bioenergetic functions of mitochondria and potential contribution to adverse health effects in non-target organisms.

Thompson, L. J., Smith, S., Stout, J. C., White, B., Zioga, E., & Stanley, D. A. (2022). Bumblebees can be Exposed to the Herbicide Glyphosate when Foraging. *Environmental Toxicology and Chemistry, 41*(10), 2603–2612.

This study researched effects resulting from the exposure of bumblebees to glyphosate during foraging. The authors found that bumblebees foraged indiscriminately on glyphosate-treated and untreated plants, with no significant difference in the overall duration of foraging behavior between the two treatments. Glyphosate residues were detected in the pollen collected by bees up to 70 hours after treatment and nectar and pollen remained present in plants for at least five days post-treatment.

Vera, M. S., & Trinelli, M. A. (2021). First evaluation of the periphyton recovery after glyphosate exposure. *Environmental Pollution, 290*, 117998.

Vera and Trinelli (2021) investigated the recovery potential of freshwater periphyton exposed to glyphosate, utilizing microcosms to simulate natural conditions. Periphyton exposed to 0.4 mg/L of glyphosate recovered most structural parameters within seven days in clean water, though the taxonomic structure did not fully revert to control conditions. However, periphyton subjected to a higher concentration of 4 mg/L glyphosate showed significant and lasting impacts, including higher proportions of dead diatoms and reduced ash-free dry weight, indicating that the effects persisted even after 21 days in glyphosate-free water.

Weidenmüller, A., Meltzer, A., Neupert, S., Schwarz, A., & Kleineidam, C. (2022). Glyphosate impairs collective thermoregulation in bumblebees. *Science, 376*(6598), 1122-1126.

This study investigates the impact of glyphosate on bumblebee thermoregulation, finding that long-term dietary exposure reduces the ability of colonies to maintain optimal brood temperatures, particularly under resource limitation. While individual thermogenesis was slightly affected, the collective thermoregulation capacity of colonies decreased by over 25%, leading to a drop in nest temperatures. The results of this study suggest that glyphosate exposure may exacerbate the effects of existing environmental stressors and consequently impact colony growth and fitness during periods of resource limitation.

Wood, L.J., Botten, N., & Werner, J.R. (2021). Glyphosate remains in forest plant tissues for a decade or more. *Forest Ecology and Management, 493*, 119259.

This study analyzed the persistence of glyphosate and its metabolite AMPA in various forest plant tissues over twelve years. Results indicated that 45% of treated samples contained glyphosate residues. Residue presence decreased exponentially, with glyphosate detection dropping from 93% to 2% and AMPA to 1% over twelve years. Fireweed roots contained glyphosate and AMPA up to twelve years post-treatment.

Yu, H., Peng, J., Cao, X., Wang, Y., Zhang, Z., & Xu, Y. (2021). Effects of microplastics and glyphosate on growth rate, morphological plasticity, photosynthesis, and oxidative stress in the aquatic species *Salvinia cucullata*. *Environmental Pollution, 279*, 116900.

The study investigated the combined ecotoxicity of polystyrene microplastics and glyphosate on *Salvinia cucullata*. The results showed that glyphosate significantly reduced the relative growth rate, photosynthetic capacity, and root activity. Microplastics did not significantly affect photosynthesis or leaf morphology but did reduce root activity. Both compounds stimulated antioxidant defenses, increasing the activity of enzymes such as superoxide dismutase and catalase. Synergistic effects, such as increased leaf yellowing, were observed at higher combined concentrations.

Zhang, W., Wang, J., Song, J., Feng, Y., Zhang, S., Wang, N., Liu, S., Song, Z., Lian, K., & Kang, W. (2021). Effects of low-concentration glyphosate and aminomethyl phosphonic acid on zebrafish embryo development. *Ecotoxicology and Environmental Safety, 226*, 112854.

In this study, the developmental toxicity of glyphosate and AMPA on zebrafish embryos was evaluated. Results showed significant reduction in survival and hatching rates and increased deformity rates when zebrafish embryos were exposed to glyphosate and AMPA for 72 hours (from 2 hours to 74 hours post-fertilization).

Zhou, Z., Ni, X., Wu, Z., & Tang, J. (2022). Physiological and transcriptomic analyses reveal the threat of herbicides glufosinate and glyphosate to the scleractinian coral *Pocillopora damicornis*. *Ecotoxicology and Environmental Safety*, *229*, 113074.

This study investigated the effects of glufosinate and glyphosate on the coral *Pocillopora damicornis* over 48 hours. Key findings included a significant increase in chlorophyll content at 24 hours post-glufosinate exposure and increased activities of superoxide dismutase and catalase (antioxidant enzymes) at 12 hours post-exposure to both herbicides. While glyphosate did not appear to induce oxidative stress following short-term exposure, it caused upregulation of genes related to glyoxylate metabolism and downregulation of genes associated with meiosis and signaling pathways in symbiotic dinoflagellates, indicating potential impacts to coral reproduction and metabolic processes.

2.5. Imazapyr

Babalola, O. O., & Van Wyk, J. H. (2021). Exposure impacts of Imazapyr formulation on larval development and thyroid histology of *Xenopus laevis*. *Environmental Science and Pollution Research, 28*(44), 50967-50974.

This study found that exposure to the imazapyr-based herbicide Arsenal at concentrations of 0.5, 2.0, and 3.5 mg/L significantly delayed the development of *Xenopus laevis* tadpoles, reduced hind-limb length, and increased whole-body mass at 3.5 mg/L compared to the control. Increased epithelial cell height was observed at all exposure levels and reduced colloidal area was observed at 0.5 and 2.0 mg/L, with thyroid gland area reduction also observed at the 2.0 mg/L dose level. These results indicate that the imazapyr-based herbicide Arsenal formulation is thyroid-active and may adversely impact metamorphic organisms.

Self, A. B., & Ezell, A. W. (2022). Control of Natural Pines Using Site Preparation Mixes with Imazapyr, Glyphosate, Saflufenacil, BAS #1, and BAS #2. In J. L. Willis, A. B. Self, & C. M. Siegert (Eds.), Proceedings of the 21st Biennial Southern Silvicultural Research Conference (pp. 72-74). U.S. Department of Agriculture Forest Service, Southern Research Station.

This study evaluated the efficacy of various site preparation tank mixtures, including imazapyr and glyphosate for controlling natural pine on a cutover forestry site in northeast Mississippi. Results indicated that glyphosate was essential for adequate pine control, with treatments containing glyphosate showing significantly higher brownout rates, reaching up to 100% at one year after treatment. While imazapyr alone did not provide sufficient pine control, its combination with glyphosate enhanced efficacy.

Yavari, S., Kamyab, H., Asadpour, R., Yavari, S., Bin Sapari, N., Baloo, L., Binti Abd Manan, T. S., Ashokkumar, V. & Chelliapan, S. (2021). The fate of imazapyr herbicide in the soil amended with carbon sorbents. *Biomass Conversion and Biorefinery, 13*, 7561-7569.

The study evaluates the impact of biochars derived from oil palm empty fruit bunches and rice husk on the behavior of imazapyr herbicide in soil. The results showed that soil amendments with biochar significantly increased the soil's sorption capacity by up to 2.34 times and reduced herbicide leaching, with oil palm empty fruit bunch biochar achieving the highest retention of imazapyr (97.1%). The photodegradation rate of imazapyr decreased in the presence of biochars, while imazapyr biodegradation was accelerated by biochar applications.

2.6. Triclopyr

Barlow, S. M., Terry, C., Gehen, S., & Corvaro, M. (2022). Developmental toxicity studies on triclopyr acid, triclopyr butoxyethyl ester, and triclopyr triethylamine salt in the rabbit. *Food and Chemical Toxicology, 161*, 112845.

This study evaluated the developmental toxicity of triclopyr acid and its variants, triclopyr butoxyethyl ester (BEE) and triclopyr triethylamine salt (TEA), in rabbits. Triclopyr acid and triclopyr TEA showed no evidence of impaired reproductive performance, fetotoxicity, or teratogenicity at maternally toxic doses. In contrast, triclopyr BEE caused increased post-implantation loss and slight skeletal anomalies at the 100 mg/kg-bw/day dose level, but only when maternal toxicity was also observed. The authors concluded that triclopyr acid and its variants are not specifically toxic to rabbit embryos and fetuses.

Barlow, S. M., Terry, C., Gehen, S., & Corvaro, M. (2022). Reproductive and developmental evaluations of triclopyr acid, triclopyr butoxyethyl ester and triclopyr triethylamine salt in the rat. *Food and Chemical Toxicology, 161*, 112806.

The study investigates the reproductive and developmental toxicity of triclopyr acid and its variants (triclopyr BEE and triclopyr TEA) in a two-generation rat study. At a dose of 250 mg/kg bw/day, significant parental toxicity was observed, including reduced body weight, feed intake, organ weight changes, and kidney toxicity. Developmental toxicity, such as reduced postnatal survival and lower body weight in F1 and F2 generations, was only noted at this high dose, likely due to maternal toxicity. Lower doses did not result in adverse effects and no specific reproductive toxicity was observed.

Curtis, A. N., & Bidart, M. G. (2021). Increased Temperature Influenced Growth and Development of *Lithobates pipiens* Tadpoles Exposed to Leachates of the Invasive Plant European Buckthorn (*Rhamnus cathartica*) and a Triclopyr Herbicide. *Environmental Toxicology and Chemistry*, 40(9), 2547-2558.

In this study, tadpoles were exposed to European buckthorn (*Rhamnus cathartica*) leachates at two temperature regimes (20°C and 25°C). After 8 weeks of exposure, warmer temperatures were associated with smaller tadpole size and increased limb bud development. Exposure to low concentrations of triclopyr had minimal effects on tadpole growth.

Voinorosky, C. L., Standen, K. M., & Stewart, K. J. (2022). Environmental impact of triclopyr on habitat quality in boreal rights-of-way. *Environmental Toxicology and Chemistry, 41*(12), 2955-2967.

This study assessed the indirect effects of triclopyr on boreal habitat quality by examining litter decomposition rates and invertebrate responses. Llitter mass loss was not significantly different within a year of treatment, but nitrogen profiles differed between treated and untreated samples. Field application rates did not affect survival and reproduction rates of *Folsomia candida*. Triclopyr concentrations causing 50% avoidance in tested invertebrates were above field application rates, suggesting minimal impact on habitat quality and ecosystem services.

3. INSECTICIDES

3.1. D-trans Allethrin

No relevant publications released from 2021 to 2023 which focused on one or more of the following topic areas were found for this chemical: biodiversity, water quality, soil health, wildlife, efficacy and effectiveness, human health and safety, and drift and effects on non-target species.

3.2. Diatomaceous Earth

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP). (2023). Safety and efficacy of the feed additive Anpro consisting of a mixture of Sepiolite and Kieselguhr (diatomaceous earth) for all terrestrial animal species. *EFSA Journal, 21*(12), e8453.

This study evaluates the safety and efficacy of Anpro, a mixture of sepiolite and kieselguhr, as a feed additive for terrestrial animals. Results indicate that ingested Anpro does not pose a dietary risk due its poor absorption, but is an inhalation hazard and a potential skin sensitizer. Efficacy data were inconclusive for all terrestrial animals, with significant reductions in aflatoxin B1 absorption observed in dairy cows and pigs, but not in chickens.

3.3. Fipronil

da Silva Pinto, T. J., Freitas, J. S., Moreira, R. A., Menezes da Silva, L. C., Cardoso Yoshii, M. P., de Palma Lopes, L. F., Veloso Goulart, B., Reghini Vanderlei, M., Badolato Athayde, D., Dreux Fraga, P., Pretti Ogura, A., Schiesari, L., Montagner, C. C., Daam, M. A., & Gaeta Espindola, E. L. (2021). Functional responses of *Hyalella meinerti* after exposure to environmentally realistic concentrations of 2,4-D, fipronil, and vinasse (individually and in mixture). *Aquatic Toxicology, 231*, 105712.

The study evaluates the impact of 2,4-D, fipronil, and vinasse on the survival, behavior, and reproduction of *Hyalella meinerti*. Results showed significant mortality in fipronil treatments and decreased swimming activity across all treatments. In addition, reproduction occurred solely in control treatments.

Eads, D., Livieri, T., Tretten, T., Hughes, J., Kaczor, N., Halsell, E., Grassel, S., Dobesh, P., Childers, E., Lucas, D., Noble, L., Vasquez, M., Grady, A. C., & Biggins, D. (2022). Assembling a safe and effective toolbox for integrated flea control and plague mitigation: Fipronil experiments with prairie dogs. *PLOS ONE*, 17(8), e0272419.

This study evaluates the use of 0.005% fipronil grain bait for controlling fleas on black-tailed prairie dogs (BTPDs) and its efficacy in plague mitigation. Fipronil grain effectively suppressed fleas on BTPDs for up to 24 months, though short-term control was variable. Fipronil grain treatments improved BTPD body condition and reproduction rates during the study period. During a plague epizootic in Colorado, BTPDs on treated habitat were protected, while those on non-treated habitat were nearly eliminated, demonstrating fipronil's effectiveness in maintaining BTPD populations and, consequently, habitat for black-footed ferrets. Despite mostly positive outcomes, the authors emphasize the need for continued research on fipronil's long-term safety and efficacy for wildlife conservation.

Farder-Gomes, C.F., Fernandes, K.M., Bernardes, R.C., Bastos, D.S.S., Oliveira, L.L., Martins, G.F., & Serrão, J.E. (2021). Harmful effects of fipronil exposure on the behavior and brain of the stingless bee *Partamona helleri* Friese (Hymenoptera: Meliponini). *Science of the Total Environment, 794*, 148678.

This study investigates the acute effects of fipronil on stingless bees, highlighting significant negative impacts on both behavior and brain health. Fipronil exposure increased the walking distance and velocity of bees threefold and twofold, respectively, compared to control bees. Increased neuron mortality markers and decreased levels of proteins necessary for neuron survival were observed via immunofluorescence analysis, suggesting the potential for brain function impairment.

Farder-Gomes, C. F., Fernandes, K. M., Bernardes, R. C., Bastos, D. S. S., Martins, G. F., & Serrão, J. E. (2021). Acute exposure to fipronil induces oxidative stress, apoptosis, and impairs epithelial homeostasis in the midgut of the stingless bee *Partamona helleri* Friese (Hymenoptera: Apidae). *Science of the Total Environment, 774*, 145679.

In this study, Farder-Gomes et al. (2021) evaluate the effects of fipronil on the midgut morphology and antioxidant activity in *Partamona helleri*. Results showed significant morphological changes and increased activities of detoxification enzymes such as superoxide dismutase, catalase, and glutathione S-transferase. Additionally, fipronil exposure was associated with increased cell death via apoptotic pathways and disrupts epithelial regeneration, indicating potential risk to the bees' midgut integrity and overall health.

Junio da Silva Pinto, T., Rocha, G. S., Moreira, R. A., Menezes da Silva, L. C., Yoshii, M. P. C., Goulart, B. V., Montagner, C. C., Daam, M. A., & Espindola, E. L. G. (2021). Multi-generational exposure to fipronil, 2,4-D, and their mixtures in *Chironomus sancticaroli*: Biochemical, individual, and population endpoints. *Environmental Pollution, 283*, 117384.

Junio da Silva Pinto et al. (2021) evaluate the multi-generational effects of fipronil and 2,4-D on *Chironomus sancticaroli*. The results showed decreased larval and pupae survival rates with both pesticides and their mixtures, while fipronil alone impaired adult survival. Additionally, exposure altered emergence times and sex ratios, with a marked reduction in egg production and hatchability.

Magnuson, J. T., Fuller, N., Huff Hartz, K. E., Anzalone, S., Whitledge, G. W., Acuña, S., Lydy, M. J., & Schlenk, D. (2022). Dietary exposure to bifenthrin and fipronil impacts swimming performance in juvenile Chinook salmon (*Oncorhynchus tshawytscha*). *Environmental Science & Technology, 56*(8), 5071-5080.

Magnuson et al. (2022) investigated the effects of dietary exposure to bifenthrin and fipronil on the swimming performance of juvenile Chinook salmon. The study found significant reductions in maximum swimming speed for salmon exposed to bifenthrin and its mixture with fipronil, but not for those exposed to fipronil alone.

Mena, F., Berrocal, S., Solano, K., Herrera, E., Gallardo, M., Jiménez, K., Aguilar, I., & Pinnock-Branford, M. (2023). Comparison of the Sensitivity of *Tetragonisca angustula* (Apidae-Meliponini) and *Apis mellifera* (Apidae-Apini) to Three Insecticides (Malathion, Imidacloprid, and Fipronil) Used in Costa Rica. *Environmental Toxicology and Chemistry*, 42(5), 1022–1031.

The study evaluated the acute toxicity and biomarker responses in the stingless bee *Tetragonisca angustula* and the honeybee *Apis mellifera* when exposed to malathion, imidacloprid, and fipronil. Fipronil showed similar toxicity to both species. The biomarkers indicated neurotoxicity (cholinesterase inhibition) and oxidative stress primarily with malathion and fipronil.

Mota Filho, T. M. M., Camargo, R. S., Zanuncio, J. C., Stefanelli, L. E. P., Matos, C. A. O., & Forti, L. C. (2021). Contamination routes and mortality of the leaf-cutting ant *Atta sexdens* (Hymenoptera: Formicidae) by the insecticides fipronil and sulfluramid through social interactions. *Pest Management Science, 77*(10), 4411-4417.

In this study, Mota Filho et al. (2021) found that fipronil and sulfluramid are dispersed among leaf-cutting ants (*Atta sexdens*) through social interactions like self-grooming, allogrooming, and touching. 100% mortality was observed after 2 days of fipronil exposure and after 21 days of sulfuramid exposure. Fipronil is noted to be active through contact and ingestion, while sulfuramid acts only thought ingestion.

Ou, Y., Yan, Z., Shi, G., Yu, Z., Cai, Y., & Ma, R. (2022). Enantioselective toxicity, degradation, and transformation of the chiral insecticide fipronil in two algae cultures. *Ecotoxicology and Environmental Safety, 235*, 113424.

The study assessed the enantioselective toxicity of fipronil on the growth of freshwater algae *Scenedesmus quaclricauda* and *Chlorella vulgaris*. R-fipronil exhibited a more significant inhibitory effect on algal growth than S-fipronil. Chlorophyll a content was more susceptible to fipronil exposure than chlorophyll b and carotenoids, showing a significant reduction in pigment concentrations. R-fipronil also showed a faster degradation rate in algae cultures, leading to the accumulation of toxic metabolites such as fipronil sulfide and sulfone. These findings highlight the substantial risk associated with fipronil use in aquatic ecosystems.

Pino-Otín, M. R., Ballestero, D., Navarro, E., Mainar, A. M., & Val, J. (2021). Effects of the insecticide fipronil in freshwater model organisms and microbial and periphyton communities. *Science of the Total Environment*, 764, 142820.

This study evaluates the toxicity of fipronil on the freshwater invertebrate *Daphnia magna*, the green algae *Chlamydomonas reinhardtii*, river periphyton, and freshwater and soil microbial communities. The median lethal concentration (LC50) for *D. magna* ranged from 0.07 to 0.38 mg/L and increased with temperature. The photosynthetic activity of *C. reinhardtii* was affected at 20°C with a median effect concentration (EC50) of 2.44 mg/L in buffer solution. For river periphyton, time-dependent toxicity was observed, with an EC50 of 0.74 mg/L. River water reduced fipronil's toxicity for both *C. reinhardtii* and periphyton, suggesting that environmental factors modulate its impact. The metabolic profiles of aquatic and soil bacteria were minimally affected.

Poché, D. M., Dawson, K., Tseveenjav, B., & Poché, R. M. (2021). Efficacy of low-dose fipronil bait against blacklegged tick (*Ixodes scapularis*) larvae feeding on white-footed mice (*Peromyscus leucopus*) under simulated field conditions. *Parasites & Vectors, 14*, 459.

Poche et al. (2021) evaluated the efficacy of a low-dose fipronil bait in controlling blacklegged tick larvae on white-footed mice under simulated field conditions. The bait was found to be palatable, with efficacy in preventing attached larvae from feeding to repletion at 100% on day

1, and decreasing to 75.2% by day 35. The bait also prevented tick molting success by 100% on day 1 and by 82.5% on day 35, with mean plasma fipronil concentrations declining over time.

Poché, D. M., Wagner, D., Green, K., Smith, Z., Hawthorne, N., Tseveenjav, B., & Poché, R. M. (2023). Development of a low-dose fipronil deer feed: Evaluation of efficacy against two medically important tick species parasitizing white-tailed deer (*Odocoileus virginianus*) under pen conditions. *Parasites & Vectors, 16*(94).

This study evaluated the efficacy of a low-dose fipronil deer feed in controlling *Ixodes scapularis* and *Amblyomma americanum* ticks on white-tailed deer. Results showed over 90% efficacy in reducing survivorship of blood-feeding female *I. scapularis*, except for a 47.2% efficacy at day 21 post-exposure in the 48-hour treatment group. For *A. americanum*, efficacy exceeded 80% in all instances, with 100% tick mortality in the 120-hour exposure group at day 7 post-exposure.

Saka, M., & Tada, N. (2021). Acute and chronic toxicity tests of systemic insecticides, four neonicotinoids and fipronil, using the tadpoles of the western clawed frog *Silurana tropicalis*. *Chemosphere, 270*, 129418.

In this study, the toxicity of four neonicotinoids (acetamiprid, clothianidin, dinotefuran, imidacloprid) and fipronil on tadpoles of the western clawed frog, *Silurana tropicalis,* was evaluated. Acute toxicity tests indicated low toxicity for the neonicotinoids and high toxicity and teratogenicity at low levels of fipronil exposure. Chronic exposure did not result in significant developmental defects or growth inhibition at environmentally relevant concentrations of any of the insecticides.

Santillán Deiú, A., Ondarza, P. M., Miglioranza, K. S. B., & de la Torre, F. R. (2021). Multibiomarker responses and bioaccumulation of fipronil in *Prochilodus lineatus* exposed to spiked sediments: Oxidative stress and antioxidant defenses. *Pesticide Biochemistry and Physiology*, 177, 104876.

This study evaluated the oxidative stress and antioxidant defenses in streaked prochilod fish (*Prochilodus lineatus)* exposed to fipronil-spiked sediments. Increased levels of lipid peroxidation and protein oxidation were observed, indicating oxidative stress in liver, gills, and brain tissues. In addition, decreased antioxidant capacity and brain acetylcholinesterase activity were reported, suggesting impacts to antioxidant defenses and neurotoxicity, respectively.

Schmitz, A., Dempewolf, S., Tan, S., Bicker, G., & Stern, M. (2021). Developmental neurotoxicity of fipronil and rotenone on a human neuronal in vitro test system. *Neurotoxicity Research*, 39(5), 1189-1202.

This study assessed the developmental neurotoxicity of fipronil and rotenone using human NT2 cells. Fipronil sulfone significantly reduced both cell viability and neurite outgrowth, but no specific developmental neurotoxicity potential was observed.

Sim, J. X. F., Drigo, B., Doolette, C. L., Vasileiadis, S., Donner, E., Karpouzas, D. G., & Lombi, E. (2023). Repeated applications of fipronil, propyzamide and flutriafol affect soil microbial functions and community composition: A laboratory-to-field assessment. *Chemosphere, 331*, 138850.

In this study, Sim et al. (2023) found that fipronil temporarily decreased the abundances of key microbial genes (e.g., ammonia monooxygenase, nitrogenase, and cellobiohydrolase), with significant recovery observed 180 days after application. In the field study, fipronil caused temporary inhibition of enzymatic activities, with recovery noted by the end of the 2-year study period. These results suggest that the effects on soil microbial activity are less persistent and more transient with compared to other pesticides studied.

Suzuki, T., Hirai, A., Khidkhan, K., Nimako, C., Ichise, T., Takeda, K., Mizukawa, H., Nakayama, S.M.M., Nomiyama, K., Hoshi, N., Maeda, M., Hirano, T., Sasaoka, K., Sasaki, N., Takiguchi, M., Ishizuka, M., Ikenaka, Y. (2021). The effects of fipronil on emotional and cognitive behaviors in mammals. *Pesticide Biochemistry and Physiology, 175*, 104847.

This study investigates the pharmacokinetic profile of fipronil in mice, dogs, and cats, and its impact on emotional and cognitive behaviors in mice. Fipronil breaks down to fipronil sulfone primarily in the liver, and both compounds have longer half-lives in dogs and cats than in mice. Behavioral tests indicate that fipronil and fipronil sulfone significantly affect emotional and cognitive behaviors, with increased dopamine levels in the striatum and serotonin levels in the hippocampus of mice.

Triques, M. C., Oliveira, D., Goulart, B. V., Montagner, C. C., Espíndola, E. L. G., & de Menezes-Oliveira, V. B. (2021). Assessing single effects of sugarcane pesticides fipronil and 2,4-D on plants and soil organisms. *Ecotoxicology and Environmental Safety, 208,* 111622.

Triques et al. (2021) investigated the long-term impacts of the herbicide 2,4-D and the insecticide fipronil on non-target terrestrial plants and soil invertebrates. Results showed that both pesticides negatively affect non-target species at concentrations lower than those recommended for pest control. For both soil types tested, *Raphanus sativus* was the most affected plant by 2,4-D, while *Folsomia candida* was the most affected soil invertebrate by fipronil. The degree of the adverse impacts was variable based on soil properties.

Williams, S.C., Linske, M.A., & Stafford, K.C. (2023). Orally delivered fipronil-laced bait reduces juvenile blacklegged tick (*Ixodes scapularis*) burdens on wild white-footed mice (*Peromyscus leucopus*). *Ticks and Tick-borne Diseases, 14(2023),* 102189.

This study evaluated the effectiveness of fipronil-laced bait in reducing juvenile blacklegged tick (*Ixodes scapularis*) burdens on white-footed mice (*Peromyscus leucopus*). The bait was readily accepted by wild *P. leucopus* and significantly reduced juvenile *I. scapularis* burdens over two years.

Wu, C.H., Lu, C.W., Hsu, T.H., Wu, W.J., & Wang, S.E. (2021). Neurotoxicity of fipronil affects sensory and motor systems in zebrafish. *Pesticide Biochemistry and Physiology,* 177, 104896.

We et al. (2021) researched how neurotoxicity caused by fipronil impacts the sensory and motor systems of zebrafish (*Danio rerio*). Results indicated a significant decrease in survival rates and reduced swimming speed and distance in adult zebrafish exposed to fipronil as well as a marked reduction in the number of lateral line hair cells in zebrafish embryos.

Zhuang, J., Jiang, Z., Chen, D., Li, J., Crabbe, M. J. C., Qiu, M., Zheng, Y., & Qu, W. (2023). Thyroid-disrupting effects of exposure to fipronil and its metabolites from drinking water based on human thyroid follicular epithelial Nthy-ori 3-1 cell lines. *Environmental Science & Technology*, 57, 6072–6084.

This study investigated the thyroid-disrupting effects of fipronil and its metabolites (FPM) on human thyroid follicular epithelial Nthy-ori 3-1 cells. Results indicated that FPM exposure activated the NRF2/KEAP1 signaling pathway, increased oxidative stress markers, and altered thyroid function-related proteins. The findings suggest that even low-dose FPM exposure can significantly disrupt thyroid cell function, highlighting potential risks to human health, particularly those concerning thyroid hormone regulation and oxidative stress.

3.4. Indoxacarb

Batista, C. H., Plata-Rueda, A., Zanuncio, J. C., Serrão, J. E., & Martínez, L. C. (2022). Indoxacarb effects on non-target predator, *Podisus distinctus* (Hemiptera: Pentatomidae). *Environmental Science and Pollution Research, 29*(29967-29975).

This study evaluates the effects of indoxacarb on the predatory stink bug *Podisus distinctus*, a non-target species in forestry pest management. Stink bugs exposed to indoxacarb for 72 hours exhibited lethality in a dose dependent manner. Indoxacarb exposure was also correlated with reduced prey consumption.

Li, Y., Liang, H., & Qiu, L. (2021). Enantioselective Bioaccumulation of the Chiral Insecticide Indoxacarb in Zebrafish (*Danio rerio*). *Environmental Toxicology and Chemistry, 40*(4), 1007-1016.

The study explored the enantioselective bioaccumulation of indoxacarb in zebrafish and found that the (-)-R-indoxacarb preferentially accumulated over (+)-S-indoxacarb. (-)-R-indoxacarb had significantly higher bioconcentration factor values and a longer half-life in zebrafish, suggesting a higher potential for risk to non-target aquatic species than (+)-S-indoxacarb.

Lin, Q., Deng, P., Feng, T., Ou, G., Mou, L., & Zhang, Y. (2023). Enantioselectivity of indoxacarb enantiomers in *Bombyx mori* larvae: toxicity, bioaccumulation and biotransformation. *Pest Management Science*, 79, 2353-2364.

In this study, the researchers developed a chiral analysis method to evaluate the toxicity, bioaccumulation, and biotransformation of indoxacarb enantiomers in *Bombyx mori* larvae. While the bioconcentration factor for R-indoxacarb trended higher than that of S-indoxacarb, S-indoxacarb was significantly more toxic and had higher biotransformation into the bioactive metabolite S-decarbomethoxyllated JW062.

Stevens, M. M., Warren, G. N., & Mo, J. (2022). Topical and dietary toxicity of emamectin benzoate, chlorantraniliprole, cyantraniliprole, and indoxacarb to larvae of the common armyworm *Mythimna convecta* (Lepidoptera: Noctuidae). *Pest Management Science, 78*(3), 1000-1007.

This study evaluates the effectiveness of four insecticides against the common armyworm, *Mythimna convecta*, in topical and dietary bioassays. While indoxacarb's effectiveness was less than emamectin benzoate and chlorantraniliprole, it showed a notable dose-response relationship via dietary exposure.

Wang, Z.-J., Wang, N.-M., Yu, Q.-T., & Xue, C.-B. (2023). Sublethal effects of an indoxacarb enantiomer insecticide on *Plutella xylostella* caterpillar and *Chrysoperla sinica* predator. *Ecotoxicology and Environmental Safety, 249*, 114400.

The study investigates the selective toxicity and sublethal effects of the indoxacarb enantiomers (+)-S-indoxacarb and (−)-R-indoxacarb on *Plutella xylostella* and *Chrysoperla sinica*. (+)-S-indoxacarb showed greater acute toxicity and significant sublethal effects on development, fecundity, and predatory ability in both species. Sublethal doses of (+)-S-indoxacarb significantly affected oviposition in *P. xylostella*, while (−)-R-indoxacarb impacted the pupal stage and adult survival of *C. sinica*.

3.5. Phenothrin

Mohsin, S. M. N., Ahmad, N., & Yusof, Y. A. (2023). Comparative efficacy trials with alcohol-added d-phenothrin formulations against *Aedes aegypti* under open-field conditions. *Pest Management Science*, 79(10), 4094-4099.

The study evaluates the efficacy of water-based d-phenothrin formulations with glycerol (D1) and propylene glycol (D2) as adjuvants against *Aedes aegypti* mosquitoes. Compared to controls, both formulations exhibited significantly higher efficacy in knockdown and mortality of adult mosquitoes, though they had minimal efficacy against immature stages of *Ae. Aegypti*. D2 was noted to be the more effective of the two formulations, achieving complete knockdown and mortality at 10 meters via cold fogging and 25 meters via thermal fogging.

3.6. Prallethrin

No relevant publications released from 2021 to 2023 which focused on one or more of the following topic areas were found for this chemical: biodiversity, water quality, soil health, wildlife, efficacy and effectiveness, human health and safety, and drift and effects on non-target species.

3.7. S-Hydroprene

No relevant publications released from 2021 to 2023 which focused on one or more of the following topic areas were found for this chemical: biodiversity, water quality, soil health, wildlife, efficacy and effectiveness, human health and safety, and drift and effects on non-target species.

3.8. Sodium Tetraborate Decahydrate

No relevant publications released from 2021 to 2023 which focused on one or more of the following topic areas were found for this chemical: biodiversity, water quality, soil health, wildlife, efficacy and effectiveness, human health and safety, and drift and effects on non-target species.

4. RODENTICIDES

4.1. Cholecalciferol

Klemann, N., Walther, B., Matuschka, F.-R., Jacob, J., & Endepols, S. (2023). The stop-feed effect of cholecalciferol (vitamin D3) and the efficacy of brodifacoum combined with cholecalciferol in Y139C-resistant Norway rats (*Rattus norvegicus*). *Journal of Pest Science, 96,* 1331-1340.

This study investigates the stop-feed effect of cholecalciferol and the efficacy of combining it with brodifacoum in managing Y139C-resistant Norway rats. While laboratory results demonstrated a significant reduction in bait consumption when cholecalciferol was added, control success still exceeded 90% in all field trials.

Noh, A. A. M., Ahmad, A. H., & Salim, H. (2023). Efficacy of cholecalciferol rodenticide to control wood rat, *Rattus tiomanicus* and its secondary poisoning impact towards barn owl, *Tyto javanica javanica*. *Scientific Reports, 13:2854*.

This study evaluated the efficacy of cholecalciferol rodenticide compared to first-generation anticoagulant rodenticides in controlling wood rats in oil palm plantations. Cholecalciferol baits resulted in a 71.39% mortality rate, which was comparable to chlorophacinone (74.20%) and significantly higher than warfarin (46.07%). Daily consumption was lowest for cholecalciferol baits at 3.03 g/day. A secondary poisoning assessment showed that barn owls consuming cholecalciferol-poisoned rats remained healthy over six months, indicating a lack of adverse effects on the owls.

5. ADJUVANTS

No relevant publications released from 2021 to 2023 which focused on one or more of the following topic areas were found for adjuvants used by the District: biodiversity, water quality, soil health, wildlife, efficacy and effectiveness, human health and safety, and drift and effects on non-target species.