Supplemental Materials 1a for the Monarch (*Danaus plexippus plexippus*) Species Status Assessment Report, Revised July 2020

U.S. Fish and Wildlife Service: Sarah Warner, Nancy Golden, Dave Warburton, Lisa Williams

The Risk of Insecticides to the Monarch Butterfly

The risk of insecticide impacts to monarchs is primarily influenced by the extent to which monarchs are exposed to insecticides throughout their range. This assessment presents an overview of: (1) the use of insecticides within monarch habitat, (2) pathways of monarch exposure to insecticides, (3) toxicity of insecticides to monarchs, and (4) a summary evaluation of insecticide risk. Factors influencing insecticide exposure and the uncertainties inherent in these factors are also presented to guide future research/monitoring and monarch conservation strategies.

Insecticides in Monarch Habitat

The monarch butterfly is widely distributed across the United States, occurring in a variety of urban and rural habitat types that include milkweed plants and other flowering forbs. Monarch habitat includes gardens and yards, urban parks, farmlands and other agricultural production areas, rights of way, and protected natural areas. Though pesticide use is most often associated with agricultural production, any habitat where monarchs are found may be subject to insecticide use or exposure. Insecticides can be used for insect pest control anywhere there is a pest outbreak or for general pest prevention. Homeowners may treat yards and gardens to protect plants from pests or purchase plants from nurseries that sell insecticide-treated plants (often from the neonicotinoid class of pesticides) as ornamentals. Natural areas, such as forests and parks, may be treated to control for insects that defoliate, bore into wood, or otherwise damage trees. Outbreaks of pests such as gypsy moths, Mormon crickets, or grasshoppers may trigger insecticide treatments over larger areas to control populations. Use of insecticides in vector control, especially pyrethroids and organophosphates, may be significant in areas of the country where mosquitoes pose a public health threat or reach nuisance levels.

Expenditures on insecticides in 2012 topped $5 billion in the United States, with 60 million pounds being used for agriculture (57%), home and garden (23%), and in the industrial/commercial/governmental sector (20%; EPA 2017). Chemical classes of the most commonly used insecticides during the time of the report (2008 - 2012) were organophosphates and carbamates, and pyrethroids (EPA 2017). In addition, neonicotinoid insecticides (a class of insecticides first registered in the 1990s) accounted for 80% of global seed treatment sales by 2008 (Jeschke et al. 2011). Treated seeds are used for nearly all of the corn and soybean crop acreage in the U.S. (Douglas and Tooker 2015), and neonicotinoid-treated plants are commonly sold as ornamentals for yards and gardens.

Given this extent of insecticide use over the wide distribution of monarch habitat across a variety of land use sectors, there is significant potential for monarchs to be exposed to insecticides in the United States.

Monarch Insecticide Exposure Pathways

Insecticide exposure pathways to both adults and larvae of the monarch include: (1) *dietary exposure* (ingestion of an insecticide on or within plant tissue that the monarch is feeding upon), and/or (2) *contact exposure* (direct contact with airborne insecticides that land on the monarch or are deposited on plants that the monarch comes in contact with). Figure 1 illustrates these potential insecticide exposure pathways to each life stage of the monarch. While the monarch may be exposed to insecticides throughout all life stages, this evaluation is limited to larval and adult stages, as these are considered to be the most significant from a biological perspective, and the most likely in actual environmental settings. Further, there are insufficient data to evaluate exposure and effects to the other life stages beyond a conceptual analysis. Due to overlapping generations of monarchs through the spring-fall months, both larvae and adults may be exposed to insecticides in any given geographic location the species may occur outside of its overwintering areas.

Figure 1. Insecticide exposure pathways to monarch life stages.

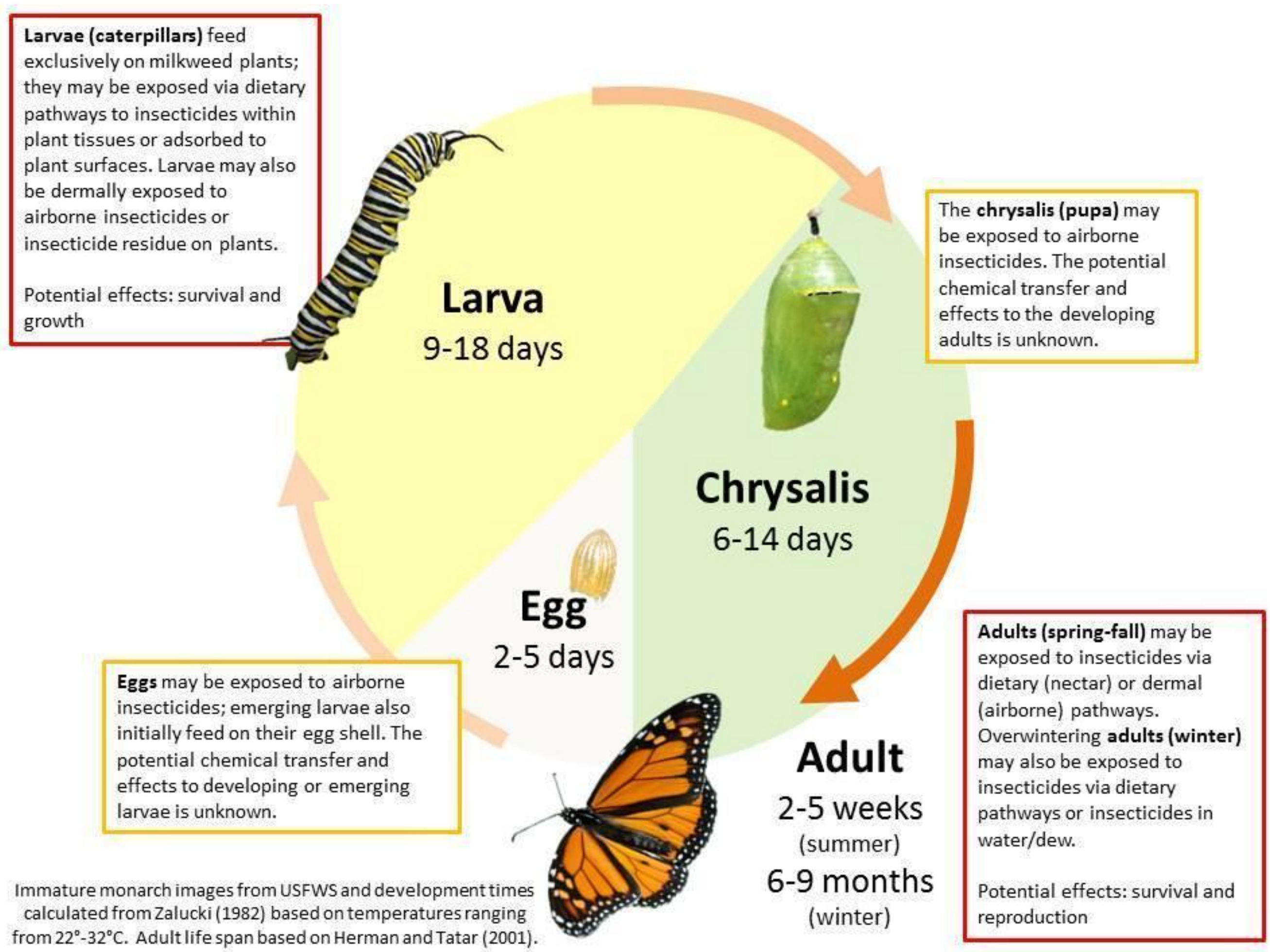

Figure produced by Kelly Nail and Dave Warburton, USFWS.

Insecticides can move through the environment and expose monarchs by the following routes:

1) **Direct Spray**: Monarchs that inhabit the same area as insect pests are susceptible to insecticide exposure (through either dietary or contact pathways) via direct spray of insecticides. One significant scenario for this occurrence is in areas subject to mosquito control with pyrethroid and organophosphate insecticides (used as mosquito adulticides).
2) **Pesticide Drift**: Monarchs may be exposed to pesticides via dietary or contact pathways in any area adjacent to a treatment location where the pesticide leaves the site of application ("drifts") via droplets, vapor, or dust. Whether a pesticide will drift, and how far from the treatment area that drift occurs, are influenced by numerous factors including method of application, height of spraying equipment, wind speed, weather conditions, nozzle size, terrain, and the use of best management practices by applicators to control for these factors and limit drift occurrences.
3) **Systemic**: Monarchs may be exposed via dietary pathways to insecticides that become

incorporated into plant tissues (e.g., leaves, pollen, nectar). Although numerous insecticides may be systemic to some degree, neonicotinoids in particular are known for this characteristic, and are expressed throughout the plant including nectar and pollen of treated crops and plants (Goulson 2013).

The degree to which an insecticide persists and moves through the environment can influence its availability, and thus exposure to monarchs. Pesticides can differ widely in these characteristics, even within the same class of chemicals; those which persist longer or are more mobile can result in greater exposure to monarchs.

For example, chemical characteristics of many neonicotinoids include high water solubility and relatively long persistence in the environment. These characteristics contribute to the propensity of neonicotinoid insecticides to transport long distances beyond use areas. Neonicotinoids have been found in well-water (Starner and Goh 2012, Huseth and Groves 2014), and can also drift off-site when incorporated into pollen (Bonmatin et al. 2015), suggesting far-reaching effects and potential landscape-scale mobility. When used as seed treatments, over 90% of the active ingredient can enter the soil and remain available (reported half-lives range from 200 to over 1000 days, Goulsen 2013). During seed sowing, less than 2% is lost in dust-off; more can be lost and deposited in the field margin areas if talcum powder or graphite is added to the seeds (Krupke et al. 2012).

For a monarch to be exposed to an insecticide through its diet, residues must be deposited on or incorporated within the dietary item associated with the relevant life stage, specifically milkweed leaves for larvae and nectar from flowers for adults. How the plant metabolizes or stores insecticides in its tissues and how it is expressed in leaves or nectar can influence exposure potential and the degree of risk to monarchs and needs to be studied. While insecticide residues have been documented in both of these media, few studies exist to help estimate concentrations (i.e., the magnitude of exposure) in the variety of areas where monarchs may be exposed, including agricultural and adjacent lands, residential areas, and parks or other presumed natural areas.

Exposure to pesticides in pollen and nectar

While monarchs are not expected to feed on pollen, reports of its widespread contamination in crop areas illustrates the ability of flowering plants to serve as sources of exposure, at least in areas in and around crops. Presence in pollen is likely indicative of presence in nectar and with further investigation into the relative accumulation of residues, concentrations measured in pollen may be used to estimate concentrations in nectar. There is some evidence that residues in nectar may be lower than those in pollen, though factors such as application method, application timing, and environmental conditions are likely to affect concentrations available to monarchs from this source. There are few North American studies measuring concentrations occurring in plants following exposure based on typical or labeled application methods, and a lack of field sampling from active crops and non-crop areas.

Investigations of contaminants in honeybee colonies illustrate that insecticides used in crops are available to pollinating insects. In a large-scale study of colonies in 23 states and one Canadian province, representing several agricultural cropping systems, concentrations of 98 different pesticides were detected in collected bee pollen (Mullin et al. 2010). Bee pollen, which aggregates pollen collected from different individuals and flowers, contained an average of 7 pesticides per sample. Chlorpyrifos was the most frequently detected insecticide in 44% of samples.

Residues of insecticides were regularly detected in pollen and nectar following two studies of experimental pesticide applications in field conditions, though concentrations varied. Average concentrations of neonicotinoids in pollen from pumpkins following various methods of application ranged up to 80.2 ng/g imidacloprid (plus an additional 19.1 ng/g metabolites), 88.3 (10.3) ng/g dinotefuran, and 95.2 (26.8) ng/g thiamethoxam (Dively and Kamel 2012). Concentrations were lower in the second year of the study, presumably due to extreme environmental conditions resulting in heat and moisture stress. Neonicotinoid metabolites accounted for 15 - 27% of total residues across years. Residues in nectar were consistently 74 - 88% lower than pollen residues, and residues in leaves were generally higher, though only correlated with values in pollen and nectar for imidacloprid. At-planting applications resulted in the lowest concentrations, and those applications occurring closer to flowering resulted in higher residues. In another study, concentrations of imidacloprid or thiamethoxam in nectar and pollen of squash treated via soil application or drip irrigation (a subset of the application methods tested in the above study) resulted in similar concentrations in pollen (5-35 ng/g) and nectar (5-20 ng/g) regardless of application method, insecticide, or study year (Stoner and Eitzer 2012). Average concentrations were 14 ng/g imidacloprid and 12 ng/g thiamethoxam in pollen, and 10 ng/g imidacloprid and 11 ng/g thiamethoxam in nectar. Residues were similar across two study years despite rainfall totals in the second year about half of those in the first. Data for metabolites were not presented.

In a study simulating greenhouse application, residues of imidacloprid and its metabolites (hydroxy and olefin), were measured in Mexican milkweed (*Asclepias curassavica*) flowers following soil applications at labeled rates for greenhouse use (Krischik et al. 2015). Whole flowers contained a mean of 6,030 ng/g midacloprid and 980 ng/g metabolites 21-51 days post-application. A second soil application 7 months after the first resulted in mean concentrations of 21,670 ng/g imidacloprid and 6,440 ng/g metabolites in whole flowers. The authors speculated that the higher residues from this application may be due to concentration in flowers during a time of slower vegetative growth. Metabolites accounted for 14% and 23% of total residues for each year, respectively, similar to the percentages measured in nectar and pollen described above. The authors acknowledge that residues in pollen and nectar may be different than residues in whole flowers and that the correlation needs to be scientifically determined.

Exposure to insecticides in milkweed leaves

Larval monarchs can be exposed to insecticides by ingesting residues that are expressed in the leaf tissue of milkweeds. Insecticides have been detected in milkweed leaves near agricultural fields in at three two studies. Variation in frequency of detection and concentration levels across years or seasons was common to both studies. While the two studies below measure concentrations in common milkweed, it is worthwhile to note that in the toxicity studies reviewed below, monarchs are exposed using four different species of milkweed plants. At present, it is not known whether the pharmacokinetics (i.e., how the plant metabolizes, stores, and expresses systemic insecticides in its tissues) is comparable across milkweed species and how this may affect the exposure and bioavailability to monarchs using these plants.

Clothianidin was measured in common milkweed (*Asclepias syriaca*) leaves that were adjacent to fields (mean distance of 1.47 m) at eight sites in South Dakota shortly after maize planting in 2014 using an ELISA method[1] (Pecenka and Lundgren 2015). Mean clothianidin concentration per plant was reported as 0.58 ppb overall and 1.14 ppb in plants with detectable residues, with a maximum 4.02 ppb in one plant. Clothianidin was detected in about half of the samples, with twice the proportion having detectable residues in July (65%) compared to June (37%). Monitoring of plants during sampling revealed that monarchs were actively using these sites, with an average of 1.3 eggs and 0.6 larvae per plant in June, and 1.4 eggs and 0.3 larvae in July.

Olaya-Arenas and Kaplan (2019) analyzed pesticides in soil and leaves of common milkweed (*Asclepias syriac*a) within 100 m of crop fields in northwest Indiana to determine if areas adjacent to fields provide greater exposure to monarchs. Three neonicotinoids were detected in leaves with variation in percent detection and concentrations by year. Clothianidin was detected in 15-25% of samples in June, but rarely detected in July or August. Concentrations varied between 2015 (0.71 ng/g mean, 56.5 ng/g maximum) and 2016 (0.48 ng/g mean, 28.5 ng/g max). Thiamethoxam was detected in just 2% of samples in 2015 (0.19 ng/g mean, 94.8 ng/g max), yet found in 75-99% in 2016 (1.87 ng/g mean, 151.3 ng/g max). Imidacloprid was only detected in 0.2% of samples in 2015 (up to 3.7 ng/g) and was not detected in 2016. The pyrethroid deltamethrin was detected in 98.9% of samples in 2016 (37.0 ng/g mean, 1,352.9 ng/g max). Distance from the edge of a crop field or the amount of crop was generally a poor predictor of pesticide detection, with only thiamethoxam demonstrating this relationship. Clothianidin was the only insecticide detected in soil, with concentrations consistent throughout the summer and correlated with those in milkweed leaves. In general, higher concentrations of insecticides were found earlier in the season with year to year variation.

Halsch et al. 2020 investigated insecticide exposure to milkweed plants across three land-use sectors that included agriculture, wildlife refuges, urban parks and gardens in northern California. The field study determined what pesticides are available to monarch during a one-time sampling event in late June - when monarch larvae are likely to be present. In this field study, 227 leaf samples of narrowleaf milkweed (*Asclepias fascicularis,*161 samples), common milkweed (*A. speciosa*, 50), woolly pod milkweed (*A. eriocarpa*, 4) and tropical milkweed (*A. curassavica*, 12) were collected from 19 sites across the Central Valley. The sites were located in conventional farms, an organic farm, a milkweed restoration site, a roadside location adjacent to an agriculture field, five in wildlife refuges, four in urban areas, and two from retail nurseries. In addition to the milkweed samples that were collected in the field, milkweed plants were purchased from home and garden stores and leaves were analyzed for pesticides. A total of 64 pesticides were detected across samples: 25 insecticides, 27 fungicides, 11 herbicides, and 1 adjuvant. A greater number of pesticides were detected in plants sampled from agricultural and retail locations compared to samples from refuge and urban sites. Chlorantraniliprole (registered for use in urban areas) was detected in 91% of the samples and methoxyfenozide (registered for

[1] In reviewing the methods as described in this paper and correspondence with one of the authors who stated that he did not think that leaf disks were weighed, it is not clear whether the reported concentrations in ppb are on a ng/g basis in the leaves or a ug/L basis in the leaf extracts, so these concentrations should be considered to be less certain than those from other publications cited in this document.

use on a variety of crops) was measured in 96% of samples. The authors compared the concentrations detected in milkweed leaves to honeybee and monarch toxicity levels. Sixteen percent (36 out of 227) of the milkweed leaves sampled had concentrations over an LD50 value for honeybee toxicity with exceedances from 7 of the 19 sampled sites. Three other pesticides (cyantraniliprole, fipronil, and methoxyfenozide) exceeded a honeybee LD50 and these were sampled from retail and urban sites. In 25% of the samples, chlorantraniliprole concentrations exceed a tested LD50 for monarchs. Clothianidin was detected above a monarch LD50 from one agriculture site. Authors indicate that for the vast majority of the pesticides detected in the milkweed leaves it is unknown what the biological effects are on monarch caterpillars.

Effects of Insecticides to Monarchs

Insecticides are pesticides with chemical properties that are designed to kill insects. Their main uses are to control insect pests in agricultural production, natural habitats, lawns and gardens, and in and around households and buildings. The U.S. Environmental Protection Agency (USEPA), under the authority of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), regulates and registers pesticides for use in the United States. To evaluate the environmental risk of proposed pesticide use as part of the registration process, the USEPA requires laboratory studies of toxicity to select non-target species. The non-native honeybee (*Apis mellifera*) is currently the primary invertebrate surrogate used in testing to evaluate risks to non-target terrestrial insects. If negative effects to non-target species are anticipated from the proposed use of a pesticide, the USEPA may choose to not approve the pesticide for registration, or to require restrictions on pesticide labels to help minimize anticipated impacts. However, under FIFRA risk management, a degree of non-target risk may be deemed acceptable if the risks are outweighed by the potential benefits of use of a pesticide. Therefore, risk to non-target species, including monarchs, cannot be ruled out simply because a pesticide has undergone the registration process and is used according to the label.

Most insecticides considered herein are non-specific and broad-spectrum in nature. That is, insects exposed to insecticides are broadly susceptible to mortality and sublethal effects. Furthermore, the larvae of many insects in the Order Lepidoptera are considered major pest species, especially in agricultural and forested areas, and insecticides are tested specifically on this taxon to ensure that they will effectively kill individuals at labeled application rates. Therefore, it is reasonable to presume that monarchs exposed to insecticides within areas of use are likely to be killed or otherwise affected following an application. Monarchs exposed in areas outside insecticide use where drift occurs may also be affected depending on the concentration of the pesticide to which they are exposed.

Scientific data documenting insecticide effects to lepidopterans are largely limited to: (1) laboratory dosing studies on larvae to investigate the toxicity of an insecticide with various endpoints measured, (2) modeling studies predicting the extent of insecticide threat to individuals or populations, and (3) field-based studies that investigate insecticide concentrations in plant tissues (as described above) and/or attempt to measure effects to populations in treated and untreated areas. All three types of studies have their limitations. For example, standardized methods of laboratory toxicity testing have not yet been adopted for lepidopteran species, resulting in inconsistencies in exposure regimes (e.g., duration, contact vs ingestion, life stage) and reporting of toxicity values (e.g., units of measurement). Lack of accepted testing protocols confound the ability to make comparisons across studies and species. Given such variability, this section presents a brief summary of select information from published literature on the effects and toxicity of three widely-used classes of insecticides to monarchs or other lepidopteran species: organophosphates, pyrethroids, and neonicotinoids. Conclusions are noted where possible. Other classes of insecticides and other types of pesticides can be similarly investigated.

Organophosphates and Pyrethroids

Information on direct toxicity of organophosphate and pyrethroid insecticides to lepidopteran species is available from efficacy studies on target pest species (particularly *Pieris brassicae* and related species, reviewed in Braak et al. 2018). In this assessment, we generally focus on toxicological effects to non-target species, with data available within the families Nymphalidae, Lycaenidae, Papilionidae, Hesperiidae, and Pieridae (Salvato 2001, Hoang et al. 2011, Eliazar and Emmel 1991, Hoang and Rand 2015, Bargar 2012a, Davis et al. 1991). Most studies measured the acute toxicity of insecticides to various lepidopteran species and report median lethal dose values (LD50s) for dietary or contact exposure pathways. Methods varied across studies in relation to length of exposure, life stage, chemical form (active ingredient vs formulated product), and exposure regime. In general, while toxicity was exhibited across all species and chemicals, no consistent patterns emerged either within or across studies that demonstrated sensitivity was related to species (or species group), life stage, or size of adults, though inconsistency in testing regimes may limit the ability to detect patterns that exist. Of the organophosphates tested (dichlorvos, malathion, naled, and dimethoate) species tended to exhibit the greatest sensitivity to naled and the least to malathion, though these results were not always consistent across species and methods. For pyrethroids, toxicity values were reported for two insecticides, permethrin and resmethrin. However, resmethrin testing was performed in formulation with piperonyl butoxide, a synergist that is combined with pesticides to enhance toxicity and comparisons cannot be made between relative toxicity of these two insecticides. Based on the available data from these insecticide studies, there is no evidence to imply that a particular species or family of lepidopterans is expected to exhibit more or less sensitivity to a particular organophosphate or pyrethroid than others, including targeted pest species.

Only two studies looked specifically at effects to monarchs within these classes of insecticides. Both studies found that monarchs exposed to pyrethroids at concentrations expected following field applications could experience mortality. Oberhauser et al. (2006) found that larvae that consumed milkweed leaves treated with permethrin in dilutions of field operable solutions (dilutions 0.5 and 0.1%) had significantly reduced rates of survival. Of the 60 larvae exposed to the two treatments, 37 died (33 as larvae and 4 as pupae) and larval stage development time was significantly delayed. Survival rates were lower for first instar larvae compared to later instar larvae. In the same study, effects to female oviposition choice, the number of eggs laid, and survival 1, 8, and 15 days after the initial spray event. Females were placed in enclosures that contained milkweeds exposed across three treatment groups: (1) milkweed plants sprayed with operational solutions of permethrin, (2) milkweed sprayed with operational solutions of permethrin, treated with oil solution, and untreated, and (3) milkweed plants that were untreated. Overall female survival was low for the two permethrin treatments (8-16 %) compared to 92% survival for the untreated treatment; with the lowest survival rate 1 day after the initial spray event. In addition, the studies found that ovipositing females did not discriminate amongst treatment groups, but fewer eggs were laid on permethrin treated plants

1 day after initial spray date compared to treated plants 8 and 15 days later.

Oberhauser et al. (2009) exposed adult and larval monarchs to ultra-low volume (ULV) applications of resmethrin (as the formulated product Scourge, which contains resmethrin plus the synergist piperonyl butoxide) to evaluate the effects of mosquito control on monarchs. Three experiments examined impacts to survival in adults and larvae subject to direct spray at varying locations upwind and downwind, and in larvae consuming previously exposed milkweed. Monarch mortality varied with conditions of experimental design, but significant increases over controls were found at distances up to 120 m downwind from the application site over the three experiments. Milkweed plants sprayed one day prior to monarch exposure resulted in significant mortality to larvae as compared to controls. In one of three experiments, adult mass was negatively affected by exposure to resmethrin. One experiment exposed house fly (*Musca domestica*) and milkweed bug (*Oncopeltus fasciatus*) larvae to resmethrin under conditions that caused monarch mortality and found no effects to survival of either species.

Neonicotinoids

There are few published studies examining the toxicity of neonicotinoids to monarchs (described herein). A summation of toxicity values of neonicotinoids across taxa (insects, birds, fish, molluscs, mammals, annelids) found insects to be the most sensitive taxa when exposed via contact or the dietary/ingestion pathway with LD50s ranging from 0.82 to 88 ng per insect (Goulson 2013). The variation in LD50 values is attributed to size of the insect, with the most sensitive insect being the brown planthopper (*Nilaparvata lugens*; a native species) weighing 1 mg, and the least sensitive insect being the Colorado potato beetle (*Leptinotarsa decemlineata*; a crop pest and non-native species) weighing 130 mg.

Three studies looked specifically at neonicotinoid effects to monarchs. While reduced survival was detected in most treatments, results of each study were influenced by differences in pesticide tested, life stage, exposure regime, and experimental methods. Pecenka and Lundgren (2015) attempted to mimic a pulsed exposure in the field by feeding swamp milkweed leaves dosed with clothianidin to larvae for 36 hours during the first stadium, and then observing effects up to the third instar. Each larva was fed a single 1 cm milkweed disk with an aqueous solution of clothianidin on agarose gel on the leaf. Once that disk was consumed, the larvae were then fed clean milkweed leaves until the end of the experiment in the third instar. Increasing mortality was observed with increasing dose, measured in µg/L (ng/g) clothianidin in the 10 µL of solution applied to each leaf disk: the LC10, LC20, LC50, and LC90 concentrations were found to be 7.72, 9.89, 15.63, and 30.70 ng/g, respectively. Significant effects to development time, body length, and weight for newly eclosed second instars were observed at doses as low as 0.5 ng/g. This study reveals effects to monarchs at seemingly low environmental concentrations of clothianidin; however, concentrations as reported (ug/L of solution per leaf disk) are not easily extrapolated to typical concentration units for a dietary testing exposure scenario (gram per leaf or ng/g ww of leaf). Therefore, it is difficult to make a direct comparison to concentrations expected to be found on milkweed leaves in the environment.

Krischik et al. (2015) investigated imidacloprid rates for greenhouse/nursery use. The authors suggest that this particular use of the insecticide can result in higher concentrations of residues found in flowering plants compared to imidacloprid used as a seed treatment; therefore, it was selected for the study. Multiple experiments were conducted using Mexican milkweed (*Asclepias curassavica)* plants with imidacloprid applied to the soil to investigate dietary exposure pathways from whole flowers or plant tissues to insects. Mexican milkweed flowers grown in soils treated with imidacloprid at labeled rates reduced survival in 3 of 4 lady beetle species, in some cases as soon as two/three days after treatment. Adult monarch and painted lady butterflies either free-ranging or force-fed imidacloprid in solution showed no effects to survival, fecundity, or egg hatch at either labeled rates or twice labeled rates. However, larval survival of both species was reduced by day 7, with few monarchs surviving past this point. Authors hypothesized that adult butterflies may not metabolize the insecticide, instead excreting it unchanged.

James (2019) examined the effects of nectar dosed with imidacloprid on monarch longevity and egg production. For the 28 day study, adult monarchs (11 males, 11 females) were consistently fed a sugar-water solution containing 23.5 ng/g imidacloprid, a concentration within the range detected in nectar of crop plants. Mortality occurred in dosed monarchs and individuals exhibited behavioral effects by day 12 (uncoordinated flapping of wings and uncontrolled vibrating of body and wings). Sample sizes throughout the study were low: At 12 days post eclosion, 4 males and 4 females remained in the dosed group with 4 males and 4 females in the control. At 22 days post eclosion, 2 individuals remained in the dosed group with 3 males and 5 females in the control. No effects were detected in mass, forewing length, oocyte development, and growth. This study tested one scenario in which adult monarchs feed on the nectar of crop plants treated with imidacloprid under certain conditions. It is uncertain the degree and frequency to which monarchs nectar on crop plants, the full range of concentrations likely to be present in treated plants, and if the imidacloprid concentration tested is representative of what could be expressed in the nectar of native flowering plants.

To determine the residue level in the milkweed tissue that leads to an adverse effect to monarchs, Barger et al. (2020) conducted three experiments that estimated the dietary exposure level of clothianidin associated with adverse effects in monarch butterflies. Results showed transfer of clothianidin from soil to milkweed plant (swamp milkweed- *Asclepias incarnata*), to larvae and to adult – this is the first study to show life-stage transfer from soil to adult. In the experiments, swamp milkweed plants were dosed (via soil treatments) with five concentrations, each experiment increasing dose levels, and larvae were exposed via dietary exposure from the time they hatched from eggs until pupation. Endpoints measured included larval survival and growth, pupation success, and adult mass. Experiment 1 consisted of concentrations that included the label rate for application of a clothianidin product, while Experiments 2 and 3 included only concentrations greater than the label application rate. In Experiment 1, clothianidin was measured in the milkweed leaves and detected in the larvae only at the two dose levels greater than the label rate, with concentrations in leaves measured at 11 ng/g (SD = 3.6) and 54 ng/g (SD = 27) in the two dose groups and in larvae at 6.0 ng/g (SD 3.3) and 13 ng/g (SD 3.4). Two of the three surviving adult butterflies from the highest dose group had detectable concentrations of clothianidin (3.1 and 5.2 ng/g). At the label application rate, concentrations in leaves, larvae, and adults were all below the detection limit and no significant effects to larval growth and survival, adult mass, pupal were observed. For Experiments 2 and 3, dose levels were all greater than label application rates for several clothianidin products. The greater dose levels resulted in detectable concentrations in leaves and larvae from all treatments. Experiment 3 was conducted to eliminate the possible effect of aphids that infested plants during Experiment 2; therefore, only the results for Experiment 3 are reported herein; however, the elevated exposure in both experiments led to adverse effects on survival and growth. Clothianidin was detected in the milkweed leaves at measurable concentrations ranging from 54 (SD = 42) to 1,545 ng/g (SD = 481), and larval consumption of the contaminated leaves negatively affected larval growth and adult survival. Larval growth was affected at 1,154 ng/g leaf and no larvae in this highest dose level reached the pupal stage. Larval mortality ranged from 50% in the lowest dose level (54 ng/g leaf) to 100% in the highest dose level, and 33-50% of the monarch butterflies died at the pupal stage in both of the lowest dose levels tested. Four adult monarchs successfully eclosed, three in the control and one in the lowest dose level. Due to the results of the three consecutive experiments, the authors suggest that clothianidin concentrations expected from applications that follow the label in wild milkweed plants are generally not high enough to adversely affect monarch butterflies and that monarchs may be relatively insensitive to clothianidin at label application rates.

Krishnan et al. 2020 conducted contact (cuticular) and dietary toxicity tests on monarch butterfly larvae at each life-stage for five insecticides that are registered for use as foliar applications on maize and soybean: a pyrethroid (beta-cyfluthrin), an anthranilic diamide (chlorantraniliprole), an organophosphate (chlorpyrifos), and two neonicotinoids (imidacloprid and thiamethoxam). For the dietary assays, larvae were reared on insecticide-treated tropical milkweed (*Asclepias curassavica*) leaves for 48 or 24 hours. Contact and dietary LD50s differed among larval stages with first instars being the most sensitive followed by third and fifth instars. The LD50 concentrations for beta-cyfluthrin and chlorantraniliprole ranged from 9.2 to 480 ng/g larva and 12.0 to 190 ng/g larva. respectively, and were the most toxic insecticides across all instars. Chlorpyrifos was the least toxic to first instars (LD50 of 79,000 ng/g larva). For the neonicotinoids, clothianidin was more toxic to larvae than both imidacloprid and thiamethoxam.

Risk Evaluation

Ecological risk assessment to evaluate potential insecticide effects to monarchs can be assessed by (1) comparing laboratory-derived toxicity values to environmental concentrations of insecticides (based on either predictive modeling or post-application sampling), and/or (2) studying effects to individuals exposed to insecticide applications in the field. Effects (lethal and sublethal) are then characterized and a determination is made as to the extent of risk. Additional (unknown) risk in the field can be caused from indirect effects of insecticides, such as susceptibility to disease or predation, and the potential for additive or even synergistic effects from exposure to multiple pesticides in the field. The lack of standardized toxicity testing andlimited monarch-specific data limit a definitive risk assessment for monarchs. Accordingly, available assessments generally center on other lepidopteran species, from which risk to monarchs can be extrapolated.

Organophosphates and pyrethroids

Though organophosphate and pyrethroid insecticides are used in all facets of pest control, there has been particular interest in performing risk assessments based on exposure scenarios from mosquito control applications, as lepidopterans can be exposed within the site of application (i.e., they occur in areas where mosquitoes are treated). In particular, the need for mosquito control in southern Florida has led to concerns regarding the effects on native lepidopterans. The few studies described below indicate that mosquito adulticide applications may pose risk to lepidopteran species, but results differed across studies, pesticide type, and species. Mosquito adulticide treatment differs from other treatments in that application rates tend to be lower than other uses, and pesticide is applied in a ULV spray designed to maximum time before deposition so as to encounter airborne mosquitoes. For these reasons, factors such as application rate and environmental transport should be considered when relating the risk assessments and field studies of mosquito adulticides described below to other uses of these insecticides (e.g., cropland, natural areas, and residential settings).

In an assessment of the risk of naled, deposition was measured 50 minutes following a single pre-dawn ULV spray for mosquito control (applied as Trumpet EC at a rate of 70 g a.i./ha; Bargar 2012b). These results were combined with morphometric data for 22 species within 5 families to estimate deposition onto butterflies roosting in the application area during a pre-dawn spray. Using lepidopteran toxicity values from the literature (described above), a 67-80% chance of exceeding the mortality estimate for the butterflies was predicted following such a spray. Assuming equivalent sensitivity, the greatest risk was estimated for butterflies within the Lycaenidae family, and the lowest risk for those within the Hesperiidae family; relative risk to butterflies within Papilionidae, Nymphalidae, and Pieridae families was considered to be intermediate.

Another risk assessment examined potential effects to native Florida caterpillars from the mosquito control pesticides permethrin, naled, and dichlorvos (Hoang and Rand 2015). Exposure data for this analysis were taken from a report generated from a field monitoring program in Big Pine Key, Florida in 2007-08, though measured values on leaves were not presented directly in Hoang and Rand (2015). The joint probability analysis in the risk assessment revealed that permethrin concentrations on host plants had a 42% chance of exceeding the lowest observed adverse effects dose (LOAED) for native Florida caterpillars and a 0.02% chance of exceeding acute LD50 values. Probabilities of exceedance for diclorovos were 11% and 2.2% for its LOAED and LD50, respectively, and the probability of exceedance was 11% for the LD50 for naled. The authors indicated that these values may underestimate actual risk in the field as they are based solely on 24-hour dietary exposure and do not consider the influence of direct topical exposure from drift or chronic exposure from insecticide persistence on leaves.

Two other field studies also examined native butterfly populations in areas with mosquito control. Population surveys in the rock pinelands of south Florida (Long Pine Key) and the Lower Florida Keys (Big Pine Key) were conducted in areas that receive year-round application of pesticides (pyrethroids and organophosphates) for mosquito control and those without such treatment (Salvato 2001). Adult densities of Florida leafwing (*Anaea troglodyta floridalis,* family Nymphalidae) were significantly lower in treated areas than in control areas. Population counts of Bartram's scrub-hairstreak (*Strymon acis bartrami*, family Lycaenidae) and Meske's skipper (*Hesperia meskei*, family Hesperiidae) did not appear to be reduced following pesticide application. In a second study, insecticide residue deposition and butterfly survival were monitored following a spray of naled during routine mosquito control in North Key Largo, Monroe County, Florida (Zhong et al. 2010). Sampling stations were set up within the spray zone, drift zone, and control areas (>25 miles away). Survival rates of 5th instar Miami blue butterfly caterpillars (*Cyclargus thomasi bethunebakeri*, family Lycaenidae) were 52-98% at sampling stations within the spray zone, and did not differ between drift and control zones. Naled was recorded in a remote drift zone 12 miles from the application area causing mortality to test mosquitoes in sampling stations, but not to butterfly larvae similarly exposed. Naled concentrations greater than 1000 ug/m^2 were associated with dramatically reduced larvae survival rates, though larvae surviving to the pupal stage successfully emerged. Wind speed was associated with higher deposition and larval mortality.

Neonicotinoids

While no field studies exist to assess the population effects of neonicotinoids, modeling studies have attempted to relate monarch declines to this class of pesticides. Forister et al. (2016) investigated neonicotinoid use and butterfly declines at four sites in Northern California that have been monitored for four decades. The model indicated an association between declining butterfly numbers and increasing neonicotinoid use, suggesting that neonicotinoids could influence populations occurring close to application sites. Similarly, Thogmartin et al. (2017) analyzed multiple threats to monarchs including climate, habitat loss, disease, and insecticides in a time series analysis using partial least squares regression models. Glyphosate and neonicotinoid use in monarch breeding habitat were both correlated with the observed monarch population decline. Gilburn et al. (2015) modeled neonicotinoid usage on agricultural lands and population estimates for 17 species of butterflies in the UK from 1985 to 2012. A negative correlation was indicated for hectares of farmland that used neonicotinoid pesticides and butterfly population declines. The authors determined that more studies are needed to determine if there is a causative link between neonicotinoid usage and the decline of butterflies, or whether the negative correlation represents a proxy for other environmental factors associated with intensive agriculture practices.

In an assessment broadly examining insecticides, DiBartolomeis et al. (2019) incorporated existing toxicity data (honeybee LD50 data for contact and oral toxicity), persistence (soil half-life), and mass applied (estimated total pounds per acre used for foliar and seed treatments) to model pesticide loading (defined as acute insecticide toxicity loading, AITL) in agricultural land and surrounding areas. The model suggests that from 1992 to 2014, the AITL in the United States increased 4-fold based on contact toxicity and 48-fold based on oral toxicity. The authors attribute this change to an increase in pesticide loading from neonicotinoids beginning in 2004. Three neonicotinoids (imidacloprid, thiamethoxam, clothianidin) combined to contribute 91.8% of the total AITL for oral toxicity. As presented, the AITL is a measure of raw insecticide toxicity in the environment and does not take into account how non-target species such as monarchs may be exposed to these chemicals. As previously discussed, factors such as accumulation in exposure media (e.g., nectar, leaf, direct spray) and the location and timing of application can be highly influential in estimating effects to individuals and populations, and may differ across classes of insecticides. Environmental persistence, as measured by a chemical's half life in soil, appears to be a significant driver in results, yet its relationship to pesticide availability to nontarget target species in unclear. As such, it is difficult to translate the conclusions of this assessment to potential effects to monarchs.

In Krishnan et al. 2020, larval dose response curves generated from toxicity studies were used to model monarch mortality rates caused by insecticide drift exposure downwind from sprayed crop fields. Two scenarios were modeled: predicted spraying for (1) soybean aphid and (2) true armyworm - a pest of maize. The models took into account three application methods: aerial application, high ground boom, and low ground boom and predicted mortality rates (using both contact and dietary larval exposure data) between 0-60 meters from the edge of a sprayed field. Application rates based on the insecticide label were used in the models. Models for aerial applications using beta-cyfluthrin and chlorantraniliprole for the soybean aphid management scenario predicted larval mortality between 100 and 32% at distances 0-60 meters downwind from the agriculture field based on cuticular toxicity data. Based on dietary toxicity data, predicted larval mortality was between 100 and 10% for modeled distances downwind from the agriculture field. Larval mortality for chlorpyrifos, imidacloprid, and thiamethoxam, (using cuticular toxicity data) was 99, 91, and 67%. For the same insecticides, larval mortality was 96, 80, and 83% based on dietary toxicity data. Modeling for high ground boom applications produced similar predictions; however, lower mortality was predicted at distances 15, 30, and 60 meters downwind compared to aerial applications in which greater larval mortality was observed at 0 meters downwind. Across the scenarios, the mortality rates were generally highest for the first instars and lowest for fifth instars. The lowest percentage of monarch mortality was modeled at 60 meters downwind from the crop edge.

Summary and Conclusions

Despite inconsistencies in testing regimes (e.g., chemical concentrations, application methods and exposure routes, and life stage and species tested), studies presented here and in other reviews (Mule et al. 2017; Braak et al. 2018) demonstrate that insecticides can have negative effects on lepidopteran species. The majority of the studies evaluated for the Monarch Species Status Assessment are laboratory toxicity tests designed to identify the insecticide concentration that causes mortality or adverse effects. More recent laboratory toxicity studies have attempted to evaluate the effects at relevant environmental concentrations. Field studies are also available that measure insecticide concentrations in milkweeds or monitor effects to lepidopterans within and outside of an application site. Finally, modeling studies weigh the risk of insecticides amongst other threats to monarch populations. Many of these studies concluded that insecticide use may potentially have negative effects to lepidopterans, including monarchs. While these studies provide pieces of information to evaluate the risk of insecticides to monarchs, enough data gaps remain for the many variables involved to prevent a comprehensive analysis of effects.

As insecticides are generally likely to cause adverse effects to butterflies, exposure of monarchs (both adults and larvae) to these chemicals through diet and contact is the primary determinant of risk across a variety of land use sectors throughout the species' range. Monarch exposure to insecticides is not readily predictable, but dependent on individual monarchs encountering pesticide residues on or near the individual plants they use. In addition, exposure is influenced by factors such as the extent and frequency of insecticide use, timing of application, application rate and method, proximity of monarchs to the application site, contact with residues in the air or on plant surfaces, availability of residues in dietary items associated with lifestage present (leaves or nectar), and pesticide persistence.

The extent and manner of insecticide use itself is not regularly monitored or easily predicted in any given area. Insecticide use can vary both temporally and spatially, and is subject to regional or broad scale changes from disease and pest outbreaks, and emerging pest pressure. The toxicity of insecticides present on the landscape to lepidopterans may change based on the development and use of new insecticides, the regulation of older insecticides, the unknown effects of pesticide mixtures in the environment, and the advent of new technologies to prevent drift and reduce nontarget exposure.

Despite the challenges to determine a quantifiable extent to which insecticides impact the monarch population, and to determine a specific cause and effect relationship of insecticide effects to monarchs in environmental settings across various land use sectors, the substantial body of information available allows for a qualitative evaluation of the risk of insecticides to monarchs. Based on insecticide chemical characteristics and use; and the exposure potential, laboratory toxicity tests, field studies, and models presented herein, *insecticides are a threat to monarch populations*. This is primarily due to insecticides being used in areas on the landscape where monarchs occur; the fact that insecticides are designed to kill insects (and in many cases specifically target lepidopteran species); insecticides are likely to cause both lethal and nonlethal effects to non-target lepidopterans that are exposed in areas of application (such as crops fields, city parks, natural areas, residential areas, and yards and gardens); and may cause both lethal and nonlethal effects to non-target insects that are exposed from drift by droplet, vapor, and dust in areas outside of application sites and from systemic incorporation into non-target plant tissues.

Though many uncertainties (described throughout this assessment) regarding insecticide exposure and effects make it difficult to determine the *degree or extent of risk* to both individuals and at the population level, there are some factors that contribute to this uncertainty that are manageable and can be addressed through conservation actions, toxicity and exposure research and methodologies, and outreach/education programs. Manageable factors include:

- General awareness of insecticide use (e.g., ornamental plants and other consumer products that may contain neonicotinoids), and public policy affecting insecticide registration and use.
- Extent of development and adoption of best management practices for insecticide use, including Integrated Pest Management (e.g., establishing “acceptable levels” of pest pressure) and drift control measures.
- Extent of agricultural land uses with monoculture systems that increase the potential for, and frequency of, insect pest outbreaks and the economic need for chemical control.
- Societal expectations for widespread use of mosquito control insecticides.
- Technological capability to develop chemical insect pest controls which are more selective for the pest species, short-lived in the environment, less mobile, etc.
- Lack of standardized toxicity testing protocols to determine effects to the monarch and other non-target lepidopterans.
- Lack of standardized methods for field studies to determine the extent of exposure to the monarch population and other non-target lepidopterans.
- Field measurements of insecticide residues in select components of monarch habitat across a variety of land use sectors (i.e., quantified exposure).
- Lack of studies that clearly relate laboratory trials and field studies to realistic field exposure and effects to monarch butterflies.

Additional research and monitoring of aspects associated with these factors can provide the information necessary to reduce the uncertainties, and to determine which factors are the most important to manage risk. Most of these factors directly relate to insecticide exposure –

managing exposure manages risk. There are several guides and references available to manage insecticide exposure as part of broader monarch conservation strategies, including:

- Monarch Butterfly Conservation Report (see page 34) https://www.fws.gov/savethemonarch/pdfs/MonarchConferenceReport2016.pdf
- USFWS IPM for Lawns and Gardens https://www.fws.gov/pollinators/pdfs/FWS_IPM_Urban_Outreach_Final_April_26_2018_final_web_508.pdf
- USFWS IPM for Farmlands https://www.fws.gov/pollinators/pdfs/FWS_IPM_Farmland_Outreach_Final_April_26_2018_web_508.pdf

Literature Cited

Barger T, Hladik ML, Daniels JC. 2020. Uptake and toxicity of clothianidin to monarch butterflies from milkweed consumption. PeerJ 8:e8669 DOI 10.7717/peerj.8669.

Bargar T. 2012a. The relationship between total cholinesterase activity and mortality in four butterfly species. Environmental Toxicology and Chemistry 31: 2124-2129.

Bargar T. 2012b. Risk assessment for adult butterflies exposed to the mosquito control pesticide naled. Environmental Toxicology and Chemistry 31:885-891.

Bijleveld M, Lexmond V, Bonmatin J, Goulson D, Noome DA. 2014. Worldwide integrated assessment on systemic pesticides Global collapse of the entomofauna: exploring the role of systemic insecticides. Environmental Science and Pollution Research 22:1-4.

Bonmatin J, Giorio C, Girolami V, Goulson D, Kreutzweiser D, Krupke C, Liess M, Long E, Marzaro M, Mitchell E, Noome D, Simon-Delso N, Tapparo A. 2015. Environmental fate and exposure; neonicotinoids and fipronil. Environmental Science and Pollution Research. 22:35-67. DOI 10.1007/s11356-014-3332-7

Braak N, Neve R, Jones AK, Gibbs M, Breuker CJ. 2018. The effects of insecticides on butterflies - A review. Environmental Pollution. 242:507-518. doi: 10.1016/j.envpol.2018.06.100.

Davis BNK, Lakhani KH, Yates TJ. 1991. The hazards of insecticides to butterflies of field margins. Agr Ecosyst Environ 36:151-161.

DiBartolomeis M, Kegley S, Mineau P, Radford R, Klein K. 2019. An assessment of acute insecticide toxicity loading (AITL) of chemical pesticides used on agricultural land in the United States.PLoS ONE 14(8): e0220029. doi.org/10.1371/journal.pone.0220029.

Dively and Kamel. 2012. Insecticide residues in pollen and nectar of a cucurbit crop and their potential exposure to pollinators. J Agric Food Chem 4449-4456.

Douglas MR, Tooker JF .2015. Large-Scale deployment of seed treatments has driven rapid increase in use of neonicotinoid insecticides and preemptive pest management in U.S. field crops. Environmental Science and Technology 49:5088-5097.

Easton AH, Goulson D. 2013. The neonicotinoid insecticide imidacloprid repels pollinating flies and beetles at field-realistic concentrations. PLoS ONE 8(1):e54819.

Eliazar PJ, Emmel TC. 1991. Adverse impacts to non-target insects. Mosquito control pesticides: ecological impacts and management alternatives. Conference Proceedings. Scientific Publishers Inc., Gainesville, Florida.

[USEPA] U.S. Environmental Protection Agency. 2017. Pesticides industry sales and usage: 2008-2012 market estimates. Office of Chemical Safety and Pollution Prevention, Washington, DC.

Forister M, Cousens B, Harrison J, Anderson K, Thorne J, Waetjen D, Nice C, De Parsia M, Hladik M, Meese R, Van Vliet H, Shapiro A. 2016. Increasing neonicotinoid use and the declining butterfly fauna of lowland California. Biological Letters 12(8):1-5.

Gilburn AS, Bunnefeld N, Wilson JM, Botham MC, Brereton TM, Fox R, Goulson D. 2015. Are neonicotinoid insecticides driving declines of widespread butterflies? PeerJ 3:el402.

Gill RJ, Ramos-Rodriguez O, Raine NE. 2012. Combined pesticide exposure severely affects individual- and colony-level traits in bees. Nature 491:105-108.

Goulson D. 2013. An overview of the environmental risks posed by neonicotinoid insecticides. Journal of Applied Ecology Ecology 50(4):977-987.

Hallmann CA, Foppen RPB, van Turnhout CAM, Kroon H de, Jongejans E. 2014. Declines in insectivorous birds are associated with high neonicotinoid concentrations. Nature 511:1-3.

Halsch C, Code A, Hoyle SM, Fordyce JA, Baert N, Forister ML, 2020. Pesticide Contamination of Milkweeds Across the Agricultural, Urban, and Open Spaces of Low-Elevation Northern California. Frontiers in Ecology and Evolution. Doi: 10.3389/fevo.2020.00162.

Henry M, Béguin M, Requier F, Rollin O, Odoux JF, Aupinel P, Aptel J, Tchamitchian S, Decourtye A. 2012. A common pesticide decreases foraging success and survival in honey bees. Science 336(6079):348–350.

Hoang T, Rand G. 2015. Acute toxicity and risk assessment of permethrin, naled, and dichlorvos to larval butterflies via ingestion of contaminated foliage. Chemosphere 120:714-721.

Hoang T, Pryor R, Rand G, Frakes R. 2011. Use of butterflies as nontarget insect test species and the acute toxicity and hazard of mosquito control insecticides. Environmental Toxicology and Chemistry 30:997-1005.

Huseth AS, Groves RL. 2014. Environmental fate of soil applied neonicotinoid insecticides in an irrigated potato agroecosystem. PLoS ONE 9(5): e97081.

James DG. 2019. A neonicotinoid insecticide at a rate found in nectar reduces longevity but not oogenesis in monarch butterflies, Danaus plexippus (L.). (Lepidoptera: Nymphalidae). Insects 10:276. doi:10.3390/insects10090276.

Jeschke P, Nauen R, Schindler M, Elbert A. 2011. Overview of the status and global strategy for neonicotinoids. Journal of Agricultural and Food Chemistry 59:2897-2908.

Krischik V, Rogers M, Gupta G, Varshney A. 2015. Soil-applied imidacloprid translocates to ornamental flowers and reduces survival of adult *Coleomegilla maculata*, *Harmonia axyridis*, and *Hippodamia convergens* lady beetles, and larval *Danaus plexippus* and *Vanessa cardui* butterflies. PLoS ONE 1-22.

Krishnan N, Zhang Y, Bidne KG, Hellmich RL, Coats JR, Bradbury SP. 2020. Assessing Field-Scale Risks of Foliar Insecticide Applications to Monarch Butterfly (Danaus plexippus) Larvae. Environmental Toxicology and Chemistry. Volume 00, Number 00—pp. 1–19.

Krupke CH, Hunt GJ, Eitzer BD, Andino G, Given K. 2012. Multiple routes of pesticide exposure for honey bees living near agricultural fields. PLoS One 7:e29268.

Mule R, Sabella G, Robba R, Manachini B. 2017. Systematic review of the effects of chemical insecticides on four common butterfly families. Frontiers in Environmental Science 5: 1-5.

Mullin CA, Frazier M, Frazier JL, Ashcraft S, Simonds R, vanEngelsdorp D, Pettis JS. 2010. High levels of miticides and agrochemicals in North American apiaries: Implications for honey bee health. PLoS ONE 5(3): e9754. https://doi.org/10.1371/journal.pone.0009754.

Oberhauser K, Brinda S, Weaver S, Moon R, Manweiler S. 2006. Growth and survival of monarch butterflies (Lepidoptera: Danaidae) after exposure to permethrin barrier treatments. Environmental Entomology 35:1626-1634.

Oberhauser K, Manweiler S, Lelich R, Blank M, Batalden R, de Anda A. 2009. Impacts of ultra-low volume resmethrin applications on non-target insects. Journal of the American Mosquito Control Association 25:83-93.

Olaya-Arenas P, Kaplan I. 2019. Quantifying pesticide exposure risk for monarch caterpillars on milkweeds boarding agricultural land. Frontiers in Ecology and Evolution 7:223. doi: 10.3389/fevo.2019.00223.

Pecenka J, Lundgren J. 2015. Non-target effects of clothianidin on monarch butterflies. Science of Nature 102:19.

Roessink I, Merga LB, Zweers HJ, Van den Brink PJ. 2013. The neonicotinoid imidacloprid shows high chronic toxicity to mayfly nymphs. Environmental Toxicology and Chemistry 32:1096-1100.

Salvato 2001. Influence of mosquito control chemicals on butterflies (Nymphalidae, Lycaenidae, Hesperiidae) of the lower Florida Keys. Journal of the Lepidopterists' Society 55: 8-14.

Starner K, Goh KS. 2012. Detections of the neonicotinoid insecticide imidacloprid in surface waters of three agricultural regions of California, USA, 2010-2011. Bulletin of Environmental Contaminants and Toxicology 88:316-321.

Stoner KA, BD Eitzer. 2012. Movement of soil-applied imidacloprid and thiamethoxam into nectar and pollen of squash (Cucurbita pepo). PLoS ONE 7(6):e39114. doi: 10.1371/journal.pone.0039114. Epub 2012 Jun 27.

Thogmartin W, Wiederholt R, Oberhauser K, Drum R, Diffendorfer J, Altizer S, Taylor O, Pleasants J, Semmens D, Semmens B, Erickson R, Libby K, Lopez-Hoffman L. 2017. Monarch butterfly population decline in North America: identifying the threatening processes. Royal Society Open Science 1-16.

Whitehorn PR, O'Connor S, Wackers FL, Goulson D. 2012. Neonicotinoid pesticide reduces bumble bee colony growth and queen production. Science 336:351–352.

Zhong H, Hribar LJ, Daniels JC, Feken MA, Brock C, Trager MD. 2010. Aerial ultra-low-volume application of naled: Impact on nontarget imperiled butterfly larvae (*Cyclargus thomasi bethunebakeri*) and efficacy against adult mosquitoes (*Aedes taeniorhynchus*). Environmental Entomology 39:1961-1972.

Supplemental Materials 1b for the Monarch (*Danaus plexippus plexippus*) Species Status Assessment Report, Revised November 2019

U.S. Fish and Wildlife Service: Sarah Warner, Nancy Golden, Dave Warburton

The Risk of Exposure and Direct Toxicity of Herbicides to the Monarch Butterfly

Herbicides are widely used throughout the range of the monarch, and can cause mortality and reduced vitality to milkweed host plants and nectar source plants. However, plants may survive exposure if the herbicide has no toxicity to the plant (e.g., it is selective only for certain plants) or if concentrations are not high enough to elicit an effect (e.g., exposure from drift). In these cases, monarch caterpillars may retain use of the host or forage plants, but may be directly exposed to herbicides through contact or diet.

As with insecticides, the potential for direct effects of herbicides on monarchs can vary by active ingredient, product additives used (e.g. surfactants), exposure pathway, life history phase exposed, timing of application, and the amount of chemical exposed to the monarch. Herbicides work by interacting with the cellular structure or biochemical pathway of the target plant, and by causing tissue damage and plant mortality. Some herbicides are enzyme inhibitors acting on the enzymes that are important for plant growth and development. Although the mode of action for herbicides is to target specific pathways for plants, there are similarities between some plant enzymes that herbicides target and insect enzymes. For example, some herbicides target acetyl CoA carboxylase in plants, an enzyme important for plant growth but also for protein synthesis in insects (Lou et al. 2001, Goldring and Read 1993). Other herbicides can target glutamine synthetase, an enzyme critical for photorespiration in plants and ammonia detoxification and reassimilation in insects (Kutlesa and Caveney 2001). For the vast majority of herbicides, the mode of action and influence on lepidopteran biological systems remains unknown.

We are unaware of published data testing the direct effects of herbicides to monarchs. This section provides an evaluation of the risks of herbicides to monarchs based on a brief summary of herbicide-lepidopteran toxicity studies; it does not include an exhaustive review of the available science.

Herbicide concentrations in milkweed leaves

As with insecticides, oral exposure of monarchs to herbicides is dependent on residues being present on or within dietary items. Olaya-Arenas and Kaplan (2019) detected herbicides in leaves of milkweed (*A. syriac*a) within 100 m of crop fields in northwest Indiana. Atrazine was the most frequently detected herbicide, in 80-87% of the samples and at the highest concentrations (2015: 6.84 ng/g mean, 0.52 ng/g median, 238.7 ng/g maximum; 2016: 37.0 ng/g mean, 4.73 ng/g median, 1352.9 ng/g maximum), followed by s-metolachlor (in greater concentrations early in season) and acetochlor.

Herbicide toxicity to lepidopterans

Studies suggest that the active ingredients in some herbicide formulations have the potential to cause lethal and sublethal effects in lepidopterans under certain exposure scenarios. Schultz et al. (2016) tested the direct effects of graminicides fluazifop-p-butyl, sethoxydim, clethodim mixed with the adjuvant NuFilm on three *Euphydryas* species in the 2nd instar larval phase under two different scenarios. In the first experiment, *E. colon* larvae were directly exposed to the treatments at labeled rates for habitat types which could be treated for invasive plants, placed in individual rearing containers, and fed until entering diapause. Control groups received a NuFilm only treatment and a water only treatment. This experiment found that contact treatment with sethoxydim reduced survivorship of pre-diapause *E. colon* larvae by 20% compared to the water only control, while there was no observed effect to larval survival from fluazifop-p-butyl, clethodim, and the NuFilm treatments. In the second experiment, all three *Euphydryas* species were exposed to fluazifop-p-butyl mixed with NuFilm; hostplants were also treated, with larvae and host plants placed within a mesocosm study design. Survival, larval development time, and feeding behavior were observed. This experiment found no effects of fluazifop-p-butyl on larval survival or development time; however, feeding group size (number of gregarious larvae) was reduced by exposure to the herbicide.

Stark et al. (2012) examined the individual effects of three formulated herbicide products containing triclopyr (Garlon 4 Ultra - a selective herbicide used to control woody plants and broad leaved plants), sethoxydim (Poast - a selective herbicide used to control grasses), and imazapyr (Stalker - a non-selective herbicide used to control grasses) directly applied to 1st instar Behr's metalmark (*Apodemia virgulti*) and their food source (buckwheat) at labeled field rates. Larvae were then fed treated plants and allowed to develop into adults. Triclopyr, sethoxydim, and imazapyr products each reduced the number of pupae (and consequently the number of adults) produced compared to the control by 24%, 27%, and 36%, respectively.

To investigate the most likely and worst case scenarios for herbicide exposure to lepidopterans, Russell and Schultz (2009) assessed the biological effects of two herbicides to the 3rd instar phase of the Puget blue (*Icaricia icarioides blackmorei*) and the cabbage white (*Pieris rapae*). The timing of the 3rd instar larval phase corresponds to when herbicides are most likely to be used in the field. Survival, development time, and growth were measured in the larvae after the exposure of two grass-specific herbicides and one surfactant (Preference) in mixtures: fluazifop-*p*-butyl and surfactant, sethoxydim and surfactant, fluazifop-*p*-butyl and water, sethoxydim and water, a water control, and an untreated control. A backpack sprayer was used to administer the treatments to simulate ground application; maximum labeled spot spraying recommended rates were applied. To test most likely scenarios, larvae were placed on host plants (*Lupinus albicaulis*) and the herbicide mixtures for each treatment were directly sprayed on the plants. Larvae were exposed to the residues via contact and dietary exposure. To test for the worst case scenario, larvae and the host plants were separately sprayed with the herbicide mixtures and the larvae were then placed on the plant to simulate maximum direct contact and dietary exposure. The study found that survival was reduced for *P. rapae* (but not for *I. i. blackmorei*) when exposed to fluazifop-*p*-butyl plus surfactant (21% reduction) and sethoxydim plus surfactant (32% reduction) compared to the control. Development time to eclosion for *I. i. blackmorei* occurred earlier in all treatment groups compared to the controls, but this was not observed for *P.*

rapae. Wing area was smaller for female *P. rapae* when exposed to fluazifop-*p*-butyl plus surfactant (10% reduction) and sethoxydim plus surfactant (14% reduction) compared to the controls. Males exhibited a 9% reduction in total wing area in the sethoxydim plus surfactant treatment.

Kutlesa and Caveney (2001) found the herbicide glufosinate-ammonium (GLA), a non-selective post-emergence contact herbicide that competitively inhibits the enzyme glutamine synthetase, to cause lethality to Brazilian skippers (*Calpodes ethlius*) from dietary exposure from concentrations calculated to be similar to field application rates. 5th instar caterpillars were placed in petri dishes on moistened filter paper and fed leaf discs from the plant species Canna lily that were treated with acute doses of GLA to determine an LD50. Each caterpillar received one treated leaf disc and were observed until it was completely consumed (approximately 24 hours) and then provisioned with untreated leaves until pupation or death. The LD50 for GLA was calculated to be slightly lower than expected residues on leaves after field application. For behavioral studies, caterpillars were fed leaves that had high and low concentrations of GLA and mass and general behaviors were recorded daily. A decline in normal activity was observed 2-3 days after treatment with a daily dose of 5 mmol and the caterpillars stopped feeding altogether after 3-4 days. Multiple normal behaviors were observed to be altered and the caterpillars died after 6-7 days after exposure.

Bohnenblust et al. (2013) did not detect toxic effects of dicamba via contact or dietary exposure to 2nd and 3rd instar larvae of the corn earworm (*Helicoverpa zea*) and the painted lady (*Vanessa cardui)*. In contact exposure studies, larvae were placed in treatments and topically dosed with dimethylamine (DMA) and diglycolamine (DGA) formulation of dicamba within a range of the field application rate and placed in individual 50-mm petri dishes. Larvae were not provisioned during the toxicity studies and mortality was assessed at 4, 8, 12, 24, and 48-hour exposure durations. Percent mortality was equal across all treatments indicating that dose had little effect on survival for both species. To assess dietary exposure on the growth and development of *H. zea* and *V. cardui* larvae, soybean (*Glycine max*) and nodding plume thistle (*Carduus nutans*) were exposed to DMA formulation using a research grade automated sprayer at four rates that represent a range of 0.0001-0.1 of the current label rate of dicamba. After spraying, plants were isolated by treatment in a greenhouse. After three days, starved larvae (24 hours with no food provisions) were placed on the treated plants (*H. zea* on soybean and *V. Cardui* on thistle) and monitored until pupation or death. No differences in *H. zea* larval survival were detected across treatments and there was no relationship detected between number of days to pupation and herbicide dose. In the tests using thistle and *V. cardui* larvae, reductions in larval and pupa mass were observed.

LaBar and Schultz (2012) did not observe lethal or sublethal effects in a field study in which the habitat of the Puget blue was sprayed with sethoxydim and a non-ionic surfactant. During observational data collections, there was little to no observed impact on larval performance in the field or on oviposition for adults in the sprayed fields compared to non-treated fields.

Summary: Risk of Herbicides to Monarchs

In the herbicide toxicity studies summarized above, results suggest that various types of herbicides may result in direct effects to lepidopterans if exposed at recommended field application rates for the labeled land use/cover type. In several studies, the simulated application site was some type of conservation area where chemical control of invasive plants was presumed, resulting in maximum exposure of herbicide to lepidopteran. It is important to note that we found no studies evaluating the effects of herbicides to lepidopterans at concentrations representative of exposure due to drift from an application site to nearby habitat (i.e., exposure concentrations at less than a maximum labeled rate) for this risk assessment.

For those herbicide-lepidopteran toxicity studies in which effects were observed, reductions in survival were generally between 20-40% of the exposed population. Effects were detected in a variety of herbicide types, including those that are non-selective, as well as those that are selective for monocots or dicots. However, results of these studies are mixed, and in a number of cases, no direct effects were found to lepidopterans from specific herbicides or particular exposure regimes.

In summary, herbicides have been detected in milkweed plants growing in proximity to agricultural fields and larval monarchs can be exposed by ingesting residues that are expressed in plant tissues; however, the direct effects of most herbicides to monarchs are unknown and likely to be highly variable. The toxicological information presented above represents a small percentage of all herbicide products used, and does not account for the most widely used herbicides such as glyphosate, atrazine, metolachlor, and 2-4 D. For those herbicides in which direct effects were detected, we are unable to elucidate the extent or specific circumstances of their use within the monarch range. While we acknowledge the potential for toxic effects of herbicides to monarchs under certain exposure conditions, we consider the effects of insecticides to be the primary driver in monarch impacts due to pesticides (insecticides, herbicides, fungicides, rodenticides, etc.).

Literature Cited

Bohnenblust E, Egan JF, Mortensen D, Tooker J. 2013. Direct and Indirect Effects of the Synthetic-Auxin Herbicide Dicamba on Two Lepidopteran Species. Environmental Entomology. 42: 586-894.

Goldring JP, Read JS. 1993. Insect acetyl-CoA Carboxylase activity during the larval, pupal, and adult stages of insect development. Comparative Biochemistry and Physiology Part B: Comparative Biochemistry 106: 855-858.

Kutlesa NJ, Caveney S. 2001. Insecticidal activity of glufosinate through glutamine depletion in a caterpillar. Pest Manag Sci 57:25–32

LaBar C, Schultz CB. 2012. Investigating the effects of grass-specific herbicides on non-target butterflies. Natural Areas Journal 32:177-189.

Lou X, Matsumoto H, Usui K. 2001. Comparison of physiological effects of fluazifop-butyl and sethoxydim on oat (*Avena sativa* L.). Weed Biology and Management 1:120-127.

Mullin, CA, Frazier M, Frazier JL, Ashcraft S, Simonds R, VanEngelsdorp D, Pettis JS. 2010. High levels of miticides and agrochemicals in North American apiaries: Implications for honey bee health. PLoS One 5, e9754.

Olaya-Arenas P, Kaplan I. 2019. Quantifying pesticide exposure risk for monarch caterpillars on milkweeds boarding agricultural land. Frontiers in Ecology and Evolution 7:223. doi: 10.3389/fevo.2019.00223.

Russell C, Schultz CB. 2009. Effects of grass-specific herbicides on butterflies: An experimental investigation to advance conservation efforts. Journal of Insect Conservation. DOI: 10.1007/s10841-009-9224-3

Schultz CB, Zemaitis JL, Cameron, Thomas M, Bowers D, Crone EE. 2016. Non-Target Effects of Grass-Specific Herbicides Differ among Species, Chemicals and Host Plants in *Euphydryas* Butterflies. Journal of Insect Conservation. October 2016, 20: 867–877.

Stark JD, Chen XD, Johnson CS. 2012. Effects of herbicides on Behr’s metalmark butterfly, a surrogate species for the endangered butterfly, Lange’s metalmark. Environmental Pollution 164: 24-27.