

**Pesticide Risk Assessment for Streaked Horned Larks: A Review of Pesticides Used in
Willamette Valley, Oregon**

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ABSTRACT

Grassland birds are declining faster than birds in any other biome likely due to land conversion, intensification of agriculture practices, and increased inputs of pesticides. The endangered species, Streaked Horned Lark, is a ground-nesting grassland bird that utilizes agriculture land to forage and breed. Willamette Valley houses the largest populations of Streaked Horned Larks within their range. Agriculture is the predominant land use type in Willamette Valley, making it crucial habitat for the persistence of the species. Though the primary threat to the larks is scarcity of large, open habitat, pesticide use may further reduce available suitable habitat. Pesticides can impact the larks through either direct or indirect means. Herbicides can decrease host plants for insects, impacting the crucial food sources for young. While insecticides negatively impact insect abundance directly. Imidacloprid, a neonicotinoid insecticide, is the most toxic pesticide used in Willamette Valley. Ingestion of treated seeds can intoxicate and even kill small passerine species with just a single dose (LD₅₀) of 41 mg/kg bw. In addition, imidacloprid is among very few chemicals that has robust research on the indirect effects to birds including decreasing crucial insects for 15 bird species, 6 of which were granivorous partially reliant on insects like Streaked Horned Larks. The altered trophic cascade resulted in declines of all 15 species. Metribuzin, 2,4-D, Bentazon, and Simazine upper LD₅₀ is within the moderately toxic range. Atrazine, Dicamba, Diuron, Dimethenamid-p, Glyphosate, and Metolachlor-s are slightly toxic to practically non-toxic. Due to limitations of available literature and data, pesticide indirect risk couldn't be quantified. Though indirect impacts are discussed when literature was present, risk was ranked only by the direct effects of pesticides. More studies must be conducted on agrochemical used in Willamette Valley to obtain a more holistic review of the risk posed to Streaked Horned Larks.

INTRODUCTION

The Streaked Horned Lark (*Eremophila alpestris strigata*) is a rare Pacific Northwest subspecies of the Horned Lark. It is federally listed as threatened under the Endangered Species Act (Pearson and Altman, 2005, Wolf et al., 2020). Habitat loss and degradation has significantly contracted the Streaked Horned Lark breeding range (Wolf et

al., 2020). Historically, the lark occurred from Southern British Columbia down through Southern Oregon (Wolf et al., 2020). Their range is now restricted to three main regions in Oregon and Washington, South Puget Lowlands in Washington, southern Washington Coast and Lower Columbia River, and Willamette Valley, Oregon (Wolf et al., 2020). In each area, the lark occurs in one of three habitat types: prairie restoration or wildlife refuge lands, airports, or agricultural land.

The Streaked Horned Lark is a ground-nesting passerine occupying sparsely vegetated, early successional grasslands (USFWS, 2021). The Willamette Valley region offers suitable habitat, primarily large grass seed fields (Moore), that most closely simulates habitat historically maintained by natural fire and flood regimes (USFWS, 2021). Anthropogenic activity has not only altered the natural disturbance cycles regulating woody and non-native vegetation, but land conversion has further reduced habitat availability (USFWS, 2021). There are approximately 420,000 acres of grass seed fields in Willamette Valley and an additional 500,000 acres of other crop types (USFWS, 2021). In the last century, native prairie and grasslands have been converted to incompatible agriculture commodities, and residential and commercial development. Willamette Valley is home to three-fourths of Oregon's total human population and is expected to nearly double in the next 50 years (USFWS, 2021), posing a threat to the largest populations of the Streaked Horned Lark within its range. As of 2019, Willamette Valley has 165 known breeding pairs, the highest number of breeding pairs relative to the three regions (USFWS, 2021). With the loss of historically suitable habitats, the agriculture land in the Valley has become critical to the survival and recovery of the lark (USFWS, 2021).

In any year, the proportion of suitable habitat may change due to fluctuating agriculture demands and variable agriculture practices such as crop rotation, fallow fields, and herbicide and pesticide application. Streaked Horned Larks move between agriculture matrixes, abandoning territories as vegetation becomes unsuitably dense, occupying new ones as conditions change (Moore). Future changes may affect the resiliency of larks in the Willamette Valley as agriculture practices influence habitat suitability and accessibility, food availability, and survival (USFWS, 2021). In fact, this has become the major concern for the preservation of many other grassland bird species in Northern Europe and North America (Boatman et al., 2004 Mineau et al., 2005, Mineau and Whiteside, 2013). Grassland

birds are experiencing the steepest declines relative to birds occupying other biomes likely due to the intensification of agriculture practices, which are highly reliant on homogenous crop matrixes and pesticides (Minaeu et al., 2005, Bartuszevige, 2001, Wolf et al., 2020, Bright et al., 2008). The lack of native vegetation and natural ecological processes, as well as land conversion of grasslands to agriculture contributes to the declines. However, the use of pesticides may also be important. Pesticide use was reported as the most common driver of grassland bird declines (Geiger et al., 2010, Hallmann et al., 2014, Poliserpi et al., 2021, Stanton et al., 2018). The larks are known to utilize agriculture habitat for nesting and foraging and are therefore likely to be exposed to harmful chemicals via direct consumption as well as chemicals remaining in soil and water, vegetation, and insects, especially during the breeding season. Pesticides within lark habitat have not largely been studied, thus this paper offers a review of agrochemicals used in the Willamette Valley that may be negatively impacting the Streaked Horned Lark.

Direct Effects of Pesticides

Direct effects of pesticides result in a reduction in bird survival and reproduction by weakening of eggshells or mortality of young or adults that may be due to one of several exposure pathways: ingestion of contaminated prey, ingestion of the pesticide product (as in the case of neonicotinoid coated seeds or surface water contamination), or topical exposure following spray application (Bright et al., 2008, Galle et al. 2009, Gibbons et al 2015). Residues may also be ingested via preening. Testing the direct effects to birds are required by the US Environmental Protection Agency (USEPA) to register a pesticide. The direct impacts are typically quantified by the acute lethal dose (LD_{50}) and chronic dietary lethal concentration (LC_{50}) for the two test species used by the EPA, bobwhite quail and mallard duck. Other common birds used to determine toxicity thresholds are grey partridges (*P. pedrix*) or pheasants.

Indirect Effects of Pesticides

Within the last two decades, concern has shifted from the direct lethal and sublethal effects on birds, to the indirect effects (Boatman et al., 2004, Bright et al., 2008, Prosser et

al., 2016). Pesticides can act indirectly on birds by reducing or even eliminating crucial host plants for arthropods, the primary food source for young during breeding season, reducing insect prey items from application of insecticides, or by depleting native, non-crop plants that serve as nest cover or food for the granivorous adult larks (Boatman et al., 2004, Posser et al., 2016, Gibbons et al., 2015, Morris et al., 2005). It is important to note that indirect effects can also result in destruction of eggs and young or adult mortality, but through trophic interactions as demonstrated in Fig. 1 (Fig. 1, Galle et al., 2009). Several studies have suggested that pesticides have adverse effects on grassland birds by reducing prey availability (Prosser et al. 2016, Boatman et al. 2004, Bright et al. 2008). Studies investigating the declines of various grassland birds, including the grey partridge and the yellowhammer (*E. citrinella*) in Europe, have demonstrated that by eliminating pesticides from agriculture field edges, habitat utilized by these species, positively impacts the abundance of invertebrates identified as prey (Prosser et al., 2016, Morris et al., 2004). Thus, pesticide-treated habitat saw lower abundances of invertebrates (Prosser et al., 2016, Morris et al., 2004). The study conducted by Morris et al. (2004) found a significant negative relationship between the weight of yellowhammer broods and the abundance of vital invertebrates. Other studies have also established that food availability is linked to reduction in success of the chicks (Boatman et al., 2004, Bright et al., 2008). Though adult Streaked Horned Larks are granivorous, insects are the primary diet of their young. Therefore, the influence of pesticides on invertebrate populations is important for the survival and persistence of Streaked Horned Lark, especially during the breeding season.

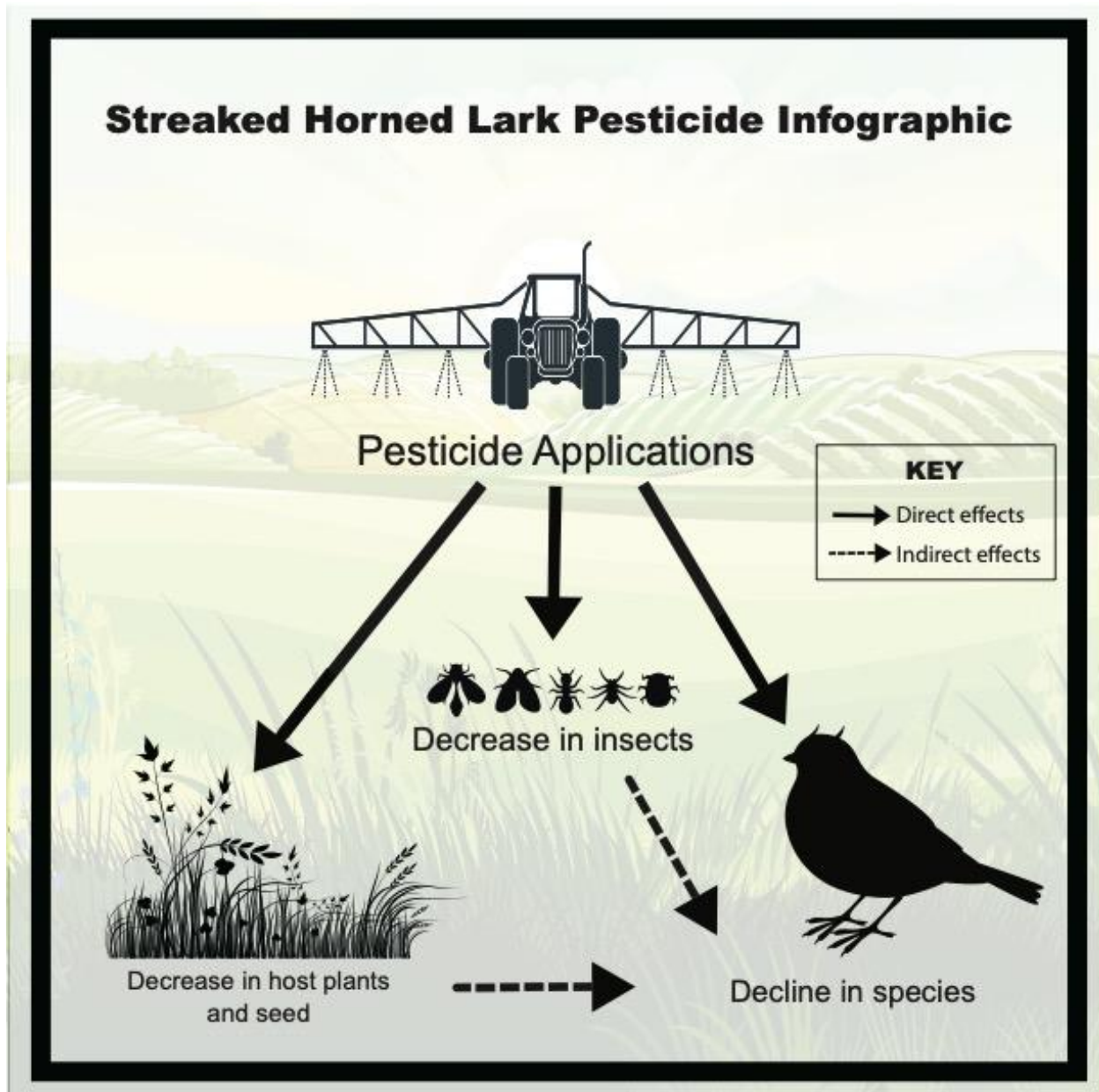


Fig. 1. Pesticides can directly (solid line) or indirectly (dashed line) impact birds. Herbicides may alter native non-crop plant structures, reducing insects and seeds for food, thus, indirectly effecting the larks. Insecticides may decrease important insects necessary for young in the breeding season. Pesticides may be ingested by birds directly, causing mortality or sublethal effects.

METHODS

Pesticide use estimates were obtained from the US Geological Survey's 2019 study, "Preliminary estimated annual agriculture pesticide use for counties of the conterminous United States" (Wieben et al 2021). Though the scope of the data was extensive, I was able to isolate the pesticide estimates of Benton, Lane, Linn, Marion, and Polk counties, which encompass the

southern Willamette Valley region. I selected these five counties as the larks are most abundant in the southern two-thirds of the Willamette Valley (Moore).

Before any pesticide can be used commercially, it must be registered with the US EPA to regulate application rates and ensure it is safe to humans and the environment. Each pesticide has a product label that outlines regulations and any restrictions that the chemical may have. I used the pesticide product labels from the US EPA for each chemical to determine the pounds applied per acre.

The USGS recorded a total of 42 chemicals across all the counties. The common chemicals used on Sod/Grass Seed crops and Grass/Pasture were determined by both The Pacific Northwest Weed Management Handbook and EPA product label documents. Thus, there are only 13 focal chemicals reviewed out of the 42 identified. In addition, chlorpyrifos has been banned in Oregon and thus, will be removed from the review.

Pesticides are often classified by their acute lethal dose (LD_{50}), measured by the mg/kg body weight (bw). LD_{50} is the quantity of pesticide that will cause death of 50% of test animals. The EPA has designated toxicity thresholds for high, moderate, low, and very low toxicity defined by LD_{50} values, as seen in Table 1 (Table 1, USDA FS 1997). All EPA registered pesticides have acute oral LD_{50} values for test bird species, commonly mallard ducks and bobwhite quail. There are very few studies done on the indirect effects of the pesticides identified in this review. In addition, indirect risk of each chemical couldn't be universally quantified across pesticides. Thus, only acute oral category was used to rank the pesticides in this review. Pesticide application estimates were not considered during the ranking progress as risk could not be definitively determined from both direct toxicity and application rates in each county.

Pesticides were categorized according to the intended target taxonomic group, either herbicides, insecticides, or fungicides, and then ranked by direct lethal dose (LD_{50}) toxicity. The pesticides that appear first in each category pose the biggest risk to Streaked Horned Larks, risk subsequently decreases.

Avian (Acute Oral):	
<i>mg/kg</i>	<i>Risk Category</i>
<10	very highly toxic
10-50	highly toxic
51-500	moderately toxic
501-2000	slightly toxic
>2000	practically non toxic

Fig 2. Acute oral risk categories used to classify toxicity to avian species.

The cropland data was available through the US Department of Agriculture National Agriculture Statistics Service cropland data layer (CDL) database (USDA-NASS 2020). The CDL is a georeferenced, raster layer developed from satellite imagery. By querying each county within the layer, I acquired the total acreage of harvested-crops and the subsequent acreage of each crop type. The latest cropland data from 2020 had data resolution of 30 meters. Crop acreages are estimated by pixel count and are not official surveys of crop acreage. Though the acreages of each crop type differed between the five Willamette Valley counties, the crops that comprised the highest percentages of area for all counties was Grass/Pasture and Sod/Grass Seed, respectively. Grass/Pasture and Sod/Grass Seed comprised 86 percent of the total agriculture crops in Benton County, 91 percent in Lane County, nearly 92 percent Linn County, 73 percent in Marion County, and 80 percent in Polk County. I used Grass/Pasture and Sod/Grass Seed crops to guide my research, emphasizing the most common chemicals used on these crop types as Streaked Horned Larks are more likely to encounter these chemicals.

I used Web of Science, Environmental Complete and Agriculture and Environmental Science Database to obtain additional literature for the review beyond the EPA product labels and registration eligibility decision documents. Each chemical is organized into direct and indirect effects based on the literature. Pesticides without the indirect effects section simply don't have any literature supporting indirect effects to birds.

RESULTS

Compound	Pesticide Type	Agriculture Type	County	High Estimate (kg)	Application Rate (lbs./acre)	Timing of Use	Direct Toxicity	Reproductive Effects	Indirect Effect Risk (found in literature)
Imidacloprid	Insecticide	Sod/Grass Seed	Marion	142	0.4	May-Mid-August	High	Yes	Yes
			Polk	76					
			Linn	30					
			Lane	21					
			Benton	2					
Metribuzin	Herbicide	Sod/Grass Seed	Marion	690	0.38-0.5	Fall	Slightly - Moderate	N/A	No
			Lane	266					
			Linn	177					
			Polk	123					
			Benton	60					
2,4-D	Herbicide	Sod/Grass Seed Grass/Pasture	Marion	6663	0.46-1.87	Spring,Fall	Practically Non-Toxic - Moderate	No	Yes
			Polk	4178					
			Linn	2895					
			Lane	2446					
			Benton	1121					
Bentazon	Herbicide	Sod/Grass Seed	Marion	2923	0.38-.05		Slightly - Moderate	N/A	No
			Linn	377					
			Benton	193					
			Polk	90					
			Lane	73					
Atrazine	Herbicide	Sod/Grass Seed Grass/Pasture	Marion	4993	0.3-2.3	Spring	Low	Yes	Yes
			Linn	2970					
			Benton	880					
			Lane	396					
			Polk	298					
Dicamba	Herbicide	Grass/Pasture	Marion	596	0.25-1	Spring	Slightly	No	Yes
			Linn	343					
			Polk	214					
			Lane	135					
			Benton	117					
Diuron	Herbicide	Sod/Grass Seed Grass/Pasture	Marion	3840	2.0-3.0 2.4	October-November 15	Slightly	N/A	No
			Polk	2078					
			Linn	1288					
			Lane	1003					
			Benton	704					
Dimethenamid	Herbicide	Sod/Grass Seed	Marion	1181	0.98	Fall,Mid-Winter	Slightly - Practically Non-Toxic	Yes	No
			Linn	563					
			Benton	343					
			Lane	120					
			Polk	94					
Simazine	Herbicide	Sod/Grass Seed	Marion	2170	1.1-2.2	Fall	Practically Non-Toxic	Yes	Yes
			Polk	676					
			Linn	442					
			Lane	332					
			Benton	219					
Glyphosate	Herbicide	Sod/Grass Seed Grass/Pasture	Marion	69668	2.09-6.26	Spring,Fall	Practically Non-Toxic	Yes	Yes
			Linn	21838					
			Polk	21034					
			Benton	17601					
			Lane	15077					
Metolachlor-s	Herbicide	Sod/Grass Seed	Marion	5760	1.04-1.38	Fall	Practically Non-Toxic	No	No
			Linn	2936					
			Benton	1237					
			Lane	692					
			Polk	479					
Azoxystrobin	Fungicide	Sod/Grass Seed	Marion	1205	0.1-0.25	Spring,Fall	Practically Non-Toxic	N/A	No
			Polk	564					
			Linn	341					
			Benton	310					
			Lane	274					
Propiconazole	Fungicide	Sod/Grass Seed	Marion	1717	0.225	Spring,Fall	Practically Non-Toxic	Yes*	No
			Polk	901					
			Linn	537					
			Lane	423					
			Benton	389					

Insecticides

Imidacloprid

Imidacloprid is the most toxic pesticide in this review. Imidacloprid was the first commercialized neonicotinoid, and its large-scale use has raised concerns about the adverse effects on non-target species (Milot et al., 2017, Hallmann et al., 2014). The adoption of seed treatments as a means of application has driven the increase in usage over the past two decades (Wood and Goulson, 2017). Neonicotinoids act by binding to the nicotinic acetylcholine receptors in the central nervous system of invertebrates (Milot et al., 2017, Hallmann et al., 2014, Tomizawa and Casida, 2004). Neonicotinoids are thought to be less harmful to vertebrates, as they have less affinity for nicotinic pathways in vertebrates relative to invertebrates (Milot et al., 2017, Hallmann et al., 2014, Tomizawa and Casida, 2004, Poliserpi et al., 2021). However, more evidence is emerging that vertebrates are negatively impacted by neonicotinoid products (Mineau and Palmer et al., 2013, Gibbons et al 2014, Roy et al., 2020). Imidacloprid, like other neonicotinoids are water-soluble and have long half-lives, and are therefore likely to accumulate in soil and ground water (Hallmann et al., 2014). Though imidacloprid is applied at lower doses relative to other pesticides, only about 5% of the chemical is taken up by the crop plants while the remainder transfers into the soil (Wood and Goulson, 2017, Eng et al., 2019). Imidacloprid can remain in soil for up to 229 days after application (Fossen, 2006). In addition, the chemical is released as dust during the seed drilling process, further increasing the quantity that disperses into the wider environment (Wood and Goulson, 2017). Thus, studies suggest that imidacloprid, and other neonicotinoids may have a significant impact on bird populations (Hallmann et al., 2014, WDFW, 2016). This is especially concerning since recommended timing for applications is within the Streaked Horned Lark breeding season, between May through mid-August (OSU, USFWS, 2021).

Imidacloprid is commonly used on Sod/Grass seed crops. There are no sources that state the use of imidacloprid, or any other neonicotinoid on pastureland. Though it is beyond the scope of this review, it is worth noting that imidacloprid is also used for cereal grain crops, which are also utilized by the larks in the Willamette Valley. Imidacloprid application estimates is highest in Marion County, followed by Polk County at 142 kg and 76 kg, respectively in 2019 (Wieben et al., 2021). The maximum dose per year is only .4 lbs/acre (.18 kg/acre) (USEPA, 2018).

Direct Effects of Imidacloprid

Though estimates appear to be low relative to other pesticides applied in Willamette Valley, neonicotinoids are moderately to highly toxic to birds on an acute oral exposure basis and slightly toxic on a subacute dietary basis (USEPA, 2012, Gibbons et al., 2015). This is especially true for small-bodied birds (Gibbons et al., 2015). The LD₅₀ value is lowest for more sensitive species such as the grey partridge that has a LD₅₀ of 14 mg/kg bw (Gibbons et al., 2015). The reported LD₅₀ value for house sparrows is 41 mg/kg bw, a similar sized species to the larks (Mineau and Palmer, 2013, Gibbons et al., 2015). Ingestion of even a few coated seeds could cause mortality or sublethal effects (Milot et al., 2017, Gibbons et al., 2015, Mineau and Palmer 2013, EPA 2020). Streaked Horned Lark adults are granivorous, thus this method of neonicotinoid treatment is identified as a threat to the populations in Willamette Valley (Milot et al., 2017).

Lopez-Anita et al (2014) gave red-legged partridges low and high doses of imidacloprid-treated seed diet. Red-legged partridges fed treated seeds with the recommended application rate for cereal crops, equal to 44 mg/kg bw, experienced a 100% mortality rate (Lopez-Anita et al., 2014). While the group that received 20% that of recommended application rate, 8.8 mg/kg bw, only saw a 18.7% mortality rate, compared to the control at 15. Still, the low doses of imidacloprid significantly reduced reproduction factors in red-legged partridges (Lopez-Anita et al., 2014). Not only did females treated with low doses of imidacloprid lay eggs later than those not dosed, treated females also had reduced clutch sizes (Lopez-Anita et al., 2014).

Mortality is more likely to occur through sublethal effects including immunological, physiological, and reproductive (Milot et al., 2017, Poliserpi et al., 2021). A study observing the neurobehavioral impact of imidacloprid sublethal dosing on South American grayish baywing (*Agelaioides badius*) found that the chemical's fast absorption caused 90% of individuals to present signs of intoxication within a half an hour after dosing (Poliserpi et al., 2021). The birds experienced reduced mobility and coordination, tremors, loss of balance, among other symptoms (Poliserpi et al., 2021, Addy-Orduna et al., 2018). A study with eared doves (*Zenaida auriculata*) measured the severity of the symptoms between dose levels. A higher percentage of individuals experienced more intense impairment with increasing doses (Addy-Orduna et al., 2018). The

most severe reactions included birds laying on the floor, half-open eyes, partial paralysis, lack of awareness, and no impulse to run or fly away (Addy-Orduna et al., 2018). Though these effects dissipate after 24 to 48 hours after sublethal dosing, the birds' ability to seek refuge, forage, or escape from a predator would be greatly compromised after consumption (Milot et al., 2017, Poliserpi et al., 2021, Eng et al., 2019, Addy-Orduna et al., 2018). Certain biomarkers indicated that dosing of imidacloprid altered brain activity, muscle tissue, and affected the liver and plasma (Poliserpi et al 2021, Eng et al. 2019). Similar neurobehavioral effects have also been recorded for rock pigeons (*Columba livia*), Japanese quails (*Coturnix japonica*), and red-winged blackbirds (*Agelaius phoeniceus*) (Bean et al., 2019, Eng et al., 2019). Sublethal effects such as ones experienced by the dosed birds may produce treated-seed avoidance (Addy-Orduna et al 2022). Though treated seed avoidance has been documented, it is a learned response, due to post-ingestion stress, not an innate aversion (Addy-Orduna et al., 2022, Lopez-Anita et al., 2014). In conjunction with the fact that birds may consume contaminated seeds in times of food scarcity, neonicotinoid seed avoidance is not sufficient to mitigate the risk of exposure and adverse effects (Mineau and Palmer, 2013, Orduna et al., 2022).

Eng et al (2019) and others have proposed that sublethal effects of neurotoxicants delay migration and reduce survival through the suppression of feeding during migratory stopover (Vyas et al., 1995). Imidacloprid exposure prevents food intake and rapid fat accumulation necessary for migrating birds (Eng 2019, Vyas et al 1995). Arriving later has adverse implications for bird survival, including decreased territory quality and offspring health (Eng 2019). Some Northern populations of Streaked Horned Larks in the Puget Sound and Washington Coast and Columbia River ecoregions migrate south to Willamette Valley for winter. Approximately 70 percent of the total lark population winter in the Valley. This is a main concern for imidacloprid application on winter wheat. Thus, application of imidacloprid in the winter, specifically on winter wheat, may be a threat to majority of the population.

Indirect Effects of Imidacloprid

Though indirect effects of neonicotinoids are difficult to measure, neonicotinoids act indirectly on grassland bird populations by suppressing the abundance of important insect prey items (Gibbons et al., 2015). Availability of food affects habitat selection, reproductive success,

chick health, and survival (Boatman et al., 2004, Morris et al., 2005, Gibbons et al., 2015, Brickle et al., 2001). Invertebrate abundances are significantly lower in agricultural fields treated with insecticides during the breeding season (Morris et al 2005, Brickle et al 2001). In the Netherlands, areas with high surface water concentrations of imidacloprid exhibited large declines of 9 insectivorous and 6 granivorous grassland bird species, all in which are reliant on a variety of insects to some extent (Hallmann et al., 2014). Within the same period, key insects, particularly Diptera (flies), Ephemeroptera (Mayfly), Odonata (dragonflies and damselflies), Coleoptera (beetles), and Hemiptera (true bugs e.g. Aphids), had sharply decreased as well (Hallmann et al., 2014). Such declines of these important invertebrate species during the breeding season reduced yellowhammer brood size due to starvation of young (Morris et al., 2005, Hallman et al., 2014). Brickle et al (2001) saw the same relationship between nest survival of corn buntings and the availability of insects close to nests. Brooding adults nesting in insecticide-treated fields must travel greater distances to forage, increasing the time in between feedings (Brickle et al., 2001). The extended time between meals negatively impacts the weight and health of chicks likely because they are fed less (Brickle et al., 2001). Moreover, the increase in energy expenditure dedicated to traveling further for food exposes adults to additional predation risk (Brickle et al., 2001).

Herbicides

Metribuzin

Effective for controlling numerous broadleaf and annual grassy weeds in warm season turfgrasses, such as Bentgrass grown for seed. Nearly 670 kg was applied to agriculture land in Marion County, followed by Linn County with 266 kg of input (Wieben et al., 2021). Though it can persist in soil and leach into surface and ground water, it is not likely to remain in water for long because metribuzin degrades rapidly in the presence of light.

Direct Effects of Metribuzin

Metribuzin is slightly to moderately toxic to birds according to available data. The acute LD₅₀ ranges from 100 to 200 mg/kg bw depending on surrogate species (OSU Extonet, 1996).

There was no further information available on chronic dietary risk or reproductive effects in avian species.

Indirect Effects of Metribuzin

Metribuzin is slightly toxic to invertebrates and thus has the potential to disrupt crucial food supplies during the breeding season. A study measuring the damage to DNA of carabid beetles from numerous pesticides found that beetles from metribuzin-treated fields had significant changes to their genome relative to beetles on organic fields (Cavaliere et al., 2019). This may indicate that metribuzin has detrimental sublethal effects to arthropods, in turn altering insect food sources for young.

2,4-D (2,4-Dichlorophenoxyacetic acid)

2,4-D is a widely used herbicide used to target broadleaf weeds in both major crop types identified in Willamette Valley, with the largest allocation to pastureland (24%) (USEPA, 2005). 2,4-D is identified as one of the most used herbicides in the Willamette Valley, with the highest estimated usage in Marion County at 6663 kg in 2019 (Wieben et al., 2021).

Direct Effects of 2,4-D

Streaked Horned Larks are ground-nesting birds that lay their eggs on bare ground, putting eggs at risk of exposure to 2,4-D. Applications on pastureland and sod/grass seed typically occur in the Spring and Fall, making exposure of eggs likely during the breeding season (USEPA, 2015). Ortiz-Santaliestra et al. (2020) conducted a study applying a conservative quantity of 2,4-D on red-legged partridge (*Alectoris rufa*) eggs to simulate spring exposure. A close to significant ($p \leq 0.069$) reduction of chick survivorship was detected from eggs sprayed with 2,4-D relative to controls (Ortiz-Santaliestra et al., 2020). This is consistent with other studies that found no variations in the hatching rate of eggs treated with varying concentrations of 2,4-D (Moran et al. 1974, Ortiz-Santaliestra et al., 2020, WHO 1989). Bird chicks saw no reproductive differences between eggs that were sprayed with 2,4-D and those that were untreated (USDA FS, 2006, Somers et al., 1978) Though survivorship of chicks isn't

significantly affected by applications of 2,4-D on eggs prior to hatching, direct oral dosing effects to birds may still be important.

The LD₅₀ acute oral values range from 472 m/kg in pheasants to 2025 mg/kg in mallard ducks (NPIC, 2008). The EPA classifies the herbicide as practically non-toxic to moderately toxic to birds on an acute oral exposure basis, based on the LD₅₀ values (NPIC, 2008). Though toxicity is relatively low, Da Silva et al. (2022) meta-analysis of the effects of 2,4-D on different organisms showed a significant increase in the mortality rate for birds when they were exposed to the herbicide. Of the studies reviewed, birds were found to be most susceptible to the lethal effects of the 2,4-D (Da Silva et al., 2022). Still, there are not many field studies that specifically outline the effects of 2,4-D application on grassland birds.

Bentazon

Bentazon is a post emergent herbicide applied to grass crops grown for seed to control broadleaf and some grassy weeds. The total Bentazon application in Willamette Valley was 3657 kg in 2019 (Wieben et al., 2021). With a half-life of just two weeks, it has a low persistence in soil. However, Bentazon has strong potential to contaminate ground and surface water as it does not bind to soil particles (OSU Exttoxnet, 1996). It is rapidly broken down in sunlight (half-life of less than 24 hours in water) thus, has a low probability of long-term contamination (OSU Exttoxnet, 1996).

Direct Effects of Bentazon

Bentazon is slightly toxic to birds on an acute and subacute basis (USEPA, 1994, OSU Exttoxnet, 1996). The oral LD₅₀ is 2000 mg/kg bw and 720 mg/kg bw for mallard ducks and Japanese quail respectively (OSU Exttoxnet, 1996). At application rates of approximately .85 lbs./acre, grass seed, grazing land, and turf crops were not found to be high acute risk to birds (EFSA, 2015).

Indirect Effects of Bentazon

Bentazon has a low risk to non-target arthropods and earthworms (EFSA, 2015). There are no additional sources that demonstrate the herbicide to disrupt the food web of grassland birds.

Atrazine

Atrazine is an herbicide applied to kill annual broadleaf weeds and grassy weeds. It may be applied to pastureland as well as perennial ryegrass and warm-season Bentgrass (USEPA, 2016). The annual average of atrazine applied to pasture is approximately 40,000 lbs. (USEPA, 2016). In addition, though beyond the scope of this review, atrazine can be used on roadsides, airfields, and wheat crops (USEPA, 2016); sites all identified as suitable habitat for Streaked Horned Larks.

Atrazine is one of the highest use estimates among all the pesticides identified in the Willamette Valley. Approximately 4993 kg was applied to Marion County agriculture land in 2019 (Wieben et al., 2021). The second highest estimate is in Linn County, with 2970 kg (Wieben et al., 2021).

Direct Effect of Atrazine

Acute toxicity is low according to the LD₅₀ for bobwhite quail, 783 mg/kg bw (USEPA, 2016). Chronic exposure of 75 mg/kg bw to mallard ducks influenced reproductive health (USEPA, 2016). A significant decrease in hatchling weight was reported for chronically dosed individuals at all concentration levels (5.3 to 12.3% at 75 to 675 mg/kg-diet) (USEPA, 2016). At concentrations at or exceeding 75 mg/kg-diet, egg production and food consumption was affected (USEPA, 2016). Bobwhite quail displayed similar side effects at 225 mg/kg-diet (USEPA, 2016). Chronic doses reduced embryo viability and egg production (USEPA, 2016). Mourning doves, eastern kingbirds, American robins, common yellowthroats, and vesper sparrows' brood quantity per season was measured in sites with atrazine application and without atrazine application. Birds in corn fields with atrazine applications had significantly lower rates of reproduction, shown by a decrease the number of broods per season, relative to control groups without exposure to atrazine (USEPA, 2016).

Indirect Effects of Atrazine

Atrazine targets broadleaf and grassy vegetation thus, may be indirectly influencing arthropod abundance by altering habitat structures on field edges and pastureland. A yearlong study conducted by Brust (1990) found that atrazine had a repellent effect on carabid beetles in greenhouse environments. Though there were no significant repellent or toxic effects of atrazine observed in fields, carabids responded to the changes in vegetation structure in treated fields (Brust, 1990). Destruction of plant material reduced the beetle abundance as it provided less suitable habitat, especially for larger beetles (Brust, 1990). This may have sizeable implications for feeding young in the breeding season.

Dicamba

Dicamba is less abundant compared to other chemicals reviewed, with the highest use in Marion County, approximately 600 kg in 2019 (Wieben et al., 2021). Dicamba is applied to both Sod/Grass Seed and Pastureland. Timing of applications can vary depending on the variety of grass; however, it is common to apply dicamba mid-fall, 4 to 6 weeks after planting grass seeds. There is not a universal recommendation for timing of applications on pastureland as rates are based on forage species and age, weed species, growth stage, in addition to other factors. Applications can occur anywhere from early spring to late fall on pasture. Moreover, it is more common in pasture to apply a mix of 2,4-D and dicamba.

Direct Effects of Dicamba

Application rates for the agriculture land of interest are between .25 to 1 lb/acre. At application rates of .3 lb./acre, no adverse effects are plausible for birds on both acute and chronic exposure basis (USFWS, 2004). However, at maximum application rates of 2 lbs/acre, reproductive effects are plausible for birds consuming contaminated insects or plant material. At dietary concentrations totaling 500 mg/kg bw per day, no reproductive effects were reported for either bobwhite quail or mallard ducks (USEPA, 2004). Acute toxicity of dicamba varies depending on test species. The LD₅₀ for pheasants is 800 mg/kg bw, while mallard ducks and bobwhite quails are less sensitive with a LD₅₀ of 1300 mg/kg (USFWS, 2004). Both values fall in the slightly toxic category.

Nevertheless, consumption of dicamba has been reported to cause intoxication even at low doses relative to the LD₅₀ values. A single oral dose of 316 mg/kg bw paralyzed domestic chickens for 19 days (USFWS, 2004). The birds were described as uncoordinated and unable to stand. The prolonged recumbency damaged the birds' sciatic nerves (USFWS, 2004). Prolonged intoxication of this nature would greatly reduce survival of wild birds.

Indirect Effects of Dicamba

A citizen science project conducted in Arkansas monitored dicamba, finding broad impacts on bird habitat from agriculture spray drift (Beyond Pesticides 2019, Scheiman 2021). In warmer weather conditions, dicamba is more likely to transition from a liquid to a gas. In this state, dicamba can rise and drift in any direction (Beyond Pesticides 2019, Scheiman 2021, Mueller and Steckel, 2021). In fact, the herbicide can move miles at a time, damaging 1.5 times more acres than formerly treated (Scheiman, 2021). Dicamba drift has landscape-scale implications, altering plant community structure far beyond agriculture applications. Consequently, impacting food availability from plants and insects that rely on host plants. Formulations of dicamba and glyphosate have become popular for managing glyphosate-resistant weeds. The addition of glyphosate to the mixture increases the concentration of dicamba in the air by three to nine times compared to the use of dicamba alone (Beyond Pesticides 2019, Mueller and Steckel, 2019). These formulations are extensively used on genetically engineered soybean and cotton crops, not suitable for larks. Still, spray drift from surrounding fields may impact vegetation structure in field-edges and suitable lark habitat, impacting food availability for Streaked Horned Larks.

Diuron

Diuron is an herbicide applied to control annual grasses and broad-leaf weeds both in Grass/Pastures and Sod/Grass Seed crops. Pastureland and seed crops in Oregon comprise the majority of diuron use compared to other states in the US (USEPA, 2003). Diuron is highly water soluble and is known to move through permeable soil into surface and ground water (USEPA 2001).

Direct Effects of Diuron

Maximum recommended application rates (6.4-12 lbs/acre) may pose potential acute risk to birds (USEPA, 2001). However, applications of diuron at such high rates are characteristic of grapes, citrus crops, and non-agriculture sites. Still, diuron application for grass seed crops, 2 to 3 lbs/acre, accumulates in the environment (USEPA, 2001). The buildup of the herbicide increases the likelihood of exposure, thus is assumed to be the most prevalent threat to reproductive impairment in birds (USEPA, 2001). In fact, as diuron biodegrades it transforms into a more toxic metabolite, 3,4-dichloroaniline. At 940 mg/kg bw, untransformed diuron is slightly toxic to bobwhite quail and practically non-toxic to mallard ducks at acute oral dose concentrations of more than 2000 mg/kg bw (USEPA, 2001). An avian reproduction study is required for diuron, however there is not study outline the risk to bird reproduction to date.

Dimethenamid-p

Dimethenamid-p is an herbicide that can either be applied as a spray or granules. Approximately 1992 kg was used in Marion County in 2019 (Wieben et al., 2021). A total of 2431 kg were applied to all of Willamette Valley (Wieben et al., 2021). Dimethenamid-P is only used on the Sod/Grass Seed crops; no literature has identified the herbicide as one used on pasture. The herbicide is moderately persistent in soil and is highly water soluble (USEPA, 2006). Thus, Dimethenamid-P has moderate risk for contaminating surface water via runoff (USEPA, 2006).

Direct Effects of Dimethenamid-P

The acute toxicity for bobwhite quail is slightly toxic with an LD₅₀ of 1,068 mg/kg bw (USEPA, 2006). On a subacute dietary basis, Dimethenamid-p is practically non-toxic at doses more than 5620 mg/kg bw. This is consistent with the findings of the European Food Safety Authority (EFSA,). The EFSA concluded a low acute and long-term risk to birds Still, lower doses had adverse physical effects on the more sensitive species, bobwhite quail. Adult male body weight significantly decreased with dietary doses at or exceeding 900 mg/kg bw (USEPA, 2006). In addition, quail eggshell thickness was reported to be significantly reduced relative to

birds with untreated diets (USEPA, 2006). However, mallard ducks did not exhibit any treatment-related effects at chronic doses less than 1800 mg/kg bw (USEPA, 2006).

The maximum treatment for grasses grown for seed is 0.98 lbs./acre. At this application rate, 20 g and 50 g birds foraging on short grasses and 20 g foraging on tall grass and broadleaf forage/small insects exceeded the EPA Acute Restricted Use and Endangered Species Level of Concern (LOC) on both an acute and dietary basis (USEPA, 2006). This risk is posed by spray and ground applications. Granule applications also exceed the Acute Risk, Restricted Use, and Endangered Species LOC at the maximum rate for the 20 g weight class. This is an important distinction as adult larks are typically between 28-48 g.

Simazine

Simazine is an herbicide that can be applied to Bentgrass, Tall and Fine Fescue, Orchardgrass, and Alfalfa crops. The recommended timing of application is in the early spring, between January and April. The highest application rate in 2019 was in Marion County (2170 kg) (Wieben et al 2021). Though the other four counties did not have large inputs of this herbicide as seen in Table (number), Marion County had a significant quantity applied. Therefore, it is important to include in this review.

Direct Effects of Simazine

Based on the LD₅₀ threshold, simazine is classified as practically non-toxic to birds on an acute exposure basis (USEPA, 2006). Though no mortality was observed during the study, birds exhibited symptoms of intoxication. Within an hour of dosing, mallard ducks had reduced response to stimuli, impaired mobility, and depression at doses exceeding 1,000 mg/kg bw (with a maximum dose of 4,640 mg/kg) (USEPA, 2006). Intoxicated individuals unable to react to perceived threats, face a greater risk of predation. A study investigating the level of contamination of atrazine in Baltic Sea animals found that birds had an accumulation of the chemical in their livers as well as magnification in their muscle tissue; magnification referring to the contaminant concentration in an organism that is higher than the concentration in their diet. Such levels likely originated from their diet of exposed fish (Reindl et al., 2015). Though the ecosystem and food chain dynamics of the Baltic Sea differ from those of agriculture settings,

the findings of this study may be analogous to exposure pathways that grassland birds may experience. Streaked Horned Larks may be exposed to simazine via contaminated food.

Indirect Effects of Simazine

Though simazine does not have a significant acute or chronic effect on carabid beetles, simazine can have repellent effects. The application of simazine deterred beetles in a greenhouse setting (Brust, 1990). Although not in a more realistic field setting, this study provides evidence of beetles negatively responding to the herbicide. This is especially concerning because the recommended application timing is in the spring growing season between January and April. A decrease in beetles and potentially other insects has consequences for lark young in the breeding season.

Glyphosate

Glyphosate is the most prominent herbicide globally and, in the US (Gill et al., 2017, Ruuskanen et al., 2020), often recognized as Roundup. It is a broad-spectrum, non-selective herbicide used to kill a variety of annual and perennial plants (Gill et al., 2017). It was identified as the top pesticide used among all counties (Wieben et al., 2021). Marion County had the highest application estimate at nearly 70,000 kg input in 2019 (Fig 2. Table 1. Wieben et al., 2021). Despite the popularity of glyphosate-based products, evidence suggests that non-target species can be exposed via accumulation in the environment as glyphosate can persist in the soil for up to a year (Ruuskanen et al., 2020, Cox, 1995). Surface and ground water can then become contaminated with increasing quantities as it leaches from the soil (Ruuskanen et al., 2020, Shehata et al., 2012). Glyphosate has a higher sorption capacity, the ability to attach to soil particles, than that of other pesticides reducing the likelihood of leaching (Gandhi et al. 2021) Still, this depends on soil composition.

This broad-spectrum herbicide used in Sod/Grass Seed, Pasture/Grass, as well as cereal crops like wheat, which are also utilized by the larks. It is estimated that up to 350 mg/kg of glyphosate can be found in treated grains (Eason and Swanlon, 2002, Ruuskanen et al., 2020). In addition to accumulation in the environment, treated grains can greatly increase the risk of exposure to Streaked Horned Larks.

Direct effects of Glyphosate

The LD₅₀ for bobwhite quail is more than 2000 mg/kg bw. With this dose level, glyphosate is classified as practically non-toxic (USEPA 1994). The direct effects of glyphosate-based products are typically non-lethal but can reduce reproductive success and physiological health. Japanese quail parents fed a sublethal concentration up to 20 mg/kg bw per day adversely effected embryo development (Ruuskanen et al., 2020). A 20 percent increase in brain lipid damage was observed in embryos from treated parents compared to the controls (Ruuskanen et al., 2020). In a separate study, Ruuskanen et al (2020) observed a low richness and abundance of beneficial gut microbes of treated Japanese quail, especially evident in young females between 12-29 weeks. The bacteria, *Lactobacillus*, known to support the immune system and gut health, decreased with cumulative glyphosate exposure, especially in developing young (Ruuskanen et al., 2020). An imbalance of gut microbes has been associated with several health issues (Simon et al., 2016, Ruuskanen et al., 2020). Glyphosate inhibits the growth of *Lactobacillus* and other gastrointestinal tract protecting bacteria have been found to be highly susceptible to glyphosate in chickens (*G. gallus*) (Shehata et al., 2012). Male birds with glyphosate diets had drastically lower testosterone levels than those not treated, both before reproductive maturity (57% decrease at 7 weeks) and after (25% decrease at 52 weeks) (Ruuskanen et al., 2020). Still, larger dietary doses of 1000 mg/kg bw are no more than slightly toxic to bobwhite quail (USEPA, 1994, USDA FS, 1997).

Indirect Effects of Glyphosate

More studies demonstrate the direct effects of glyphosate than potential indirect effects on grassland birds. Still, herbicides are designed to destroy weeds, even crucial plants that serve as food and shelter for insects (Cox, 1995, Gandhi et al., 2021). Treatment of the glyphosate-based product, Roundup, in a Maine clear-cut caused an 89% reduction in abundance of plant-eating insects (Cox, 1995). Moreover, glyphosate reduces the abundance of seed-producing plants in field-margins that are important source of food for birds, such as the granivorous larks (Prosser et al., 2016). In a separate study, glyphosate treated fields significantly decreased the population of large beetles compared to untreated plots (Brust, 1990). In fact, the beetles did not return to the treated plots until approximately a month after the application (Brust, 1990). This

may have repercussions for lark chicks. A reduction in food resources caused by herbicides in agriculture field-margin reduces production and survival of chicks (Prosser et al., 2016).

Metolachlor-s

Metolachlor-s is among the top highest application rate in Willamette Valley, 5760 kg and 2936 kg in Marion County and Lane County respectively (Wieben et al., 2021). This herbicide persistent in surface soil for up to 292 days, with a half-life minimum of 7 days (USEPA, 1995). Metolachlor-s can be highly mobile in soil, depending on soil composition (USEPA, 1995). Residues of metolachlor have been detected in ground water and surface water (USEPA, 1995). It is also among the top chemicals found in surface water in locations with high treatment rates.

Direct Effects of Metolachlor-S

The acute lethal dose (LD₅₀) is 4640 mg/kg bw for mallard ducks (USEPA, 1995). According to the LD₅₀ value, metolachlor-s is practically non-toxic to birds. This is also true for subacute dietary exposure scenarios (USEPA, 1995). Application on short grass at 6 lbs/acre exceed the EPA's acceptable LOC for endangered birds. However, the application rates for grass seed crops are much lower. The risk LOC for application rates at 2 lbs./acre or less do not exceed the EPA chronic risk threshold (USEPA, 1995).

Fungicides

Azoxystrobin

Azoxystrobin is a fungicide that can be applied to grass crops grown for seed. Marion County applied approximately 1205 kg of the fungicide in 2019 (Wieben et al 2021). Though azoxystrobin can degrade rapidly in sunlight (half-life of 11 days), the fungicide has been documented to be moderately persistent in soil (half-life of 72 to 164 days) (USEPA, 1997). It binds to larger particles of soil, while having a less affinity for binding to finer, moist soils (USEPA, 1997). Thus, the chemical can be mobile in the environment and have a potential for polluting surface and ground water. Still, there is low risk of soil and environmental persistence under realistic conditions (USEPA, 1997).

Direct Effects of Azoxystrobin

This chemical is classified as practically nontoxic to birds with a LD₅₀ of more than 2000 mg/kg bw for bobwhite quail and more than 250 mg/kg for mallard ducks. It is practically nontoxic for these species on a subacute dietary basis as well. The application on turf does not exceed the acute LOC for birds when considering the maximum number of applications (USEPA, 1997). There are no available data on the effects on avian reproduction.

Propiconazole

Propiconazole is a fungicide used on Sod/Grass Seed crops to target fungi. There is no literature stating that this propiconazole is applied to Pasture/Grass land. Estimates range from approximately 1717 kg in Marion County to 390 kg in Benton County (Wieben et al 2021).

Direct Effects of Propiconazole

At application rates of .225 lb./acre, the risk associated with grass crops grown for seed are not above the EPA's Acute Restricted level of concern (USEPA, 2006). The LD₅₀ value for the bobwhite quail is 2825 mg/kg bw, classifying propiconazole as practically non-toxic (USEPA 2006). At a dose of 1000 mg/kg bw, bobwhite quail had no treatment-related effects on a chronic basis (USEPA 2006). Dietary exposure poses low acute and low long-term risk to birds. Therefore, the EPA does not have a concern for chronic risk to birds.

Though chronic risk to birds is low, studies have demonstrated physiological responses to the chemical. Ronis et al (1994) administered 400 mg/kg bw of propiconazole to male bobwhite quail once a day for three days. Propiconazole inhibited cytochrome P450 enzymes in the treated bobwhite quail; enzymes responsible for steroid hormone regulation (Ronis et al., 1994, Marty et al., 2017). Changes to steroid hormone levels may exert behavioral effects on stress, courtship, and aggression (Steinman and Trainor, 2010).

DISCUSSION

Marion County has the largest input of pesticides compared to the other counties of Willamette Valley. Thus, Streaked Horned Lark populations located in Marion County have a

greater risk of exposure to pesticides. Metribuzin, 2,4-D, Bentazon, and Simazine upper LD₅₀ is within the moderately toxic range. Atrazine, Dicamba, Diuron, Dimethenamid-p, Glyphosate, and Metolachlor-s are slightly toxic to practically non-toxic. Imidacloprid is the most toxic pesticides identified in this review, potentially posing the largest threat to the larks due to its direct toxicity and indirect disruptions in agrosystem food webs. The seed treatments also increase the probability that larks will consume the insecticide. Still, herbicides applied at higher rates, such as 2,4-D and glyphosate, have a strong potential to indirectly effect larks. However, there is a lack of research quantifying the indirect impacts of herbicides and other pesticides on non-target species. Direct consumptive effects are emphasized and may be underestimating the actual danger of agrochemicals (Prosser et al., 2016). Only a few surrogate species are used to represent other bird species. In addition, there are multiple chemicals that the EPA requires avian reproductive reports or additional avian studies but have not since conducted such studies. More studies must be conducted to assess direct and indirect effects of pesticides to wild birds (Prosser et al., 2016, USEPA, 2004).

There is little information about the exact chemicals exposed to the larks due to uncertainty of pesticide application relative to lark populations. Zinc phosphide, commonly used by farmers and by airports to control rodent populations, was not identified in the US Geological survey. However, its highly toxic nature poses a threat to larks in Willamette Valley. Zinc phosphide has been found in the gizzard of a dead Streaked Horned Larks at an airport in Corvallis, Oregon (ABC 2019). Like neonicotinoids, just a few treated granules are enough to kill a bird (ABC 2019). The acute LD₅₀ is only 47.2 mg/kg bw for Horned Larks, classifying it as a Category I, highly toxic pesticide. Still, the application rates for zinc phosphide are not known in Willamette Valley and must be evaluated.

Mitigation measures can be implemented to reduce impacts on birds. The presence of spilled imidacloprid-treated seed piles, though rare, have been reported in some incidents of bird poisonings in France (Milot et al 2016). To protect wild birds, treated seeds should be incorporated into soil and seed spills should be removed. In addition, pesticides with liquid formulations should have specific spray drift mitigation requirements. Though these practices may reduce exposure, decreasing overall pesticide use is the most effective method of mitigation.

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