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Ecological Risk Assessment for European Grapevine Moth Control in Nurseries and Noncommercial Applications

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Introduction

The purpose of this screening level environmental risk assessment is to quantify the potential risks of APHIS-funded program activities for the eradication of the European grapevine moth (EGVM) to nontarget organisms, including fish and wildlife. Currently, commercial growers are voluntarily making insecticide treatments in vineyards; however, there may be a need to treat host material outside of these areas. Residential areas where grapes may by grown, or natural areas where wild grapes can occur, may require treatment to insure these areas do not become sources for re-infestation of commercial vineyards. The preferred treatment option in these non-commercial areas is the removal of host fruit/flowers or host plants. In cases where host plant or fruit removal is not possible, APHIS, in cooperation with the California Department of Food and Agriculture (CDFA), may treat these areas with a dispenser pheromone formulation and/or a treatment with one of three foliar insecticides. The products that may be used in these situations are ground based treatments of the microbial insecticide, *Bacillus thuringiensis* var. *kurstaki* (Btk), spinosad, or methoxyfenozide for treatments that are currently labeled for use. These treatments will be made within a 500-meter radius of any positive EGVM trap detection (USDA–APHIS, 2010). These chemistries, as well as chlorantraniliprole, may also be used in production nurseries that ship live grapevines and olive plants. This is a minor part of the overall eradication program since there are not a large number of these types of nurseries, and they usually ship dormant plants that would not require chemical treatment. However, treatments may occur in nurseries if nondormant plants are shipped from areas under quarantine.

The below assessment provides a characterization of the risk of these treatments to nontarget organisms by conducting an effects and exposure analysis, and then integrating the two to characterize direct and indirect risk to nontarget organisms. This assessment provides a characterization of the response data available from published literature, databases, and other source material. The response data consists of dose-response studies used to establish effect thresholds, such as median lethal dose (LC/LD_{50}) values, as well as sublethal endpoints, such as no observable effect levels/concentrations (NOEL(C)) or lowest observable effect levels/concentrations (LOEL(C)). Other nonstandardized laboratory and field study results were also incorporated into this risk assessment, where appropriate.

The exposure analysis provides an overview of the environmental fate of each pesticide, as well as estimates of potential exposure. Exposure estimates in terrestrial and aquatic environments are based on maximum labeled rates for each pesticide in grapes and/or ornamental applications that are designed to create upper bound estimates of exposure that would not be expected to occur under more realistic program applications. The risk characterization is an integration of the effects and exposure analysis to determine if there is the potential for risk. For terrestrial vertebrates, this was done by comparing potential doses on various food items based on a range of different types of vertebrates and then dividing those values by the specific acute or chronic effect endpoint for different types of vertebrates to estimate a risk quotient (RQ). Values greater than one using sublethal endpoints, such as NOELs, were presumed to mean that there is a potential for adverse risk to a population. This approach is not as conservative when using median lethality values (LD_{50}); therefore, further discussion regarding the RQ is warranted to discuss the potential for direct acute risk. In this screening assessment, aquatic risk was determined by looking at the range of effects data for aquatic organisms and the range of exposure concentrations estimated using modeling to determine if there was overlap which

would suggest potential for aquatic risk. In cases where no overlap occurs between effects data and exposure residues, there was a presumption that adverse risk to aquatic populations would not be expected. In cases where overlap does occur, additional discussion regarding the potential for risk is addressed.

EGVM Pheromone Dispenser

The EGVM pheromone belongs to a group of compounds known as straight chain lepidopteran pheromones (SCLP) that serve as a chemical cue attracting male moths to females of the same species for reproduction. Lepidopteran pheromones are a unique mixture of short chain hydrocarbons, similar to fatty acids, with one of several functional groups (i.e., acetate, alcohol, aldehyde). In the case of EGVM, the female emits a pheromone blend that has been identified as (E,Z)-7,9-Dodecadien-1-yl acetate, which is the primary constituent that provides species specificity to ensure attraction of the male EGVM for reproduction (Roelofs et al., 1973; Morse and Meighen, 1986; El-Sayed et al., 1999; Witzgall et al., 2005). The identification and synthesis of these types of pheromones have been successfully used as a means to provide species-specific suppression of target insect populations, including leafrollers, such as EGVM (Suckling and Shaw, 1992; Suckling and Shaw, 1995; Suckling et al., 2007; Carde and Minks, 1995; Plettner, 2002; Welter et al., 2005; Witzgall et al., 2008). This type of insect control acts by releasing a synthetic version of the naturally produced pheromone into the atmosphere which can reduce reproduction by either creating false plumes that male moths will follow, mask, or camouflage the natural plumes released by the female moths, or through decreased sensitivity of male moths to the pheromone due to high background concentrations (Stelinski, 2007). Release of synthetic pheromone into target areas will be implemented using the formulation Isomate® EGVM, which is a dispenser that contains the EVGM pheromone (figure 1). The dispenser is composed of a plastic polyethylene tube filled with the pheromone and an aluminum wire. Dispensers are attached to a tree or other object using the wire by hand at a rate of 200 dispensers per acre (equivalent to 38.36 g ai/acre) where the EGVM pheromone can volatilize into the atmosphere for approximately 120 to 180 days before removal and possible replacement. The active ingredient, or pheromone, comprises approximately 75.68% of the formulation. Due to the instability of the pheromone in the presence of light and oxygen, stabilizers are added to the pheromone formulation. The additional materials added to the formulation are certified organic by the U.S. Department of Agriculture (USDA), National Organic Program (NOP). Many of the dispenser formulations are also certified by the Organic Materials Review Institute (OMRI), and the EGVM formulation is approved for organic use (Pacific Biocontrol, 2010).

Due to similarities in fate and effects of SCLP, regulatory agencies have adopted a structure activity relationship approach in their registration (Weatherston and Minks, 1995). Current registration data requirements for SCLP in the United States, Canada, and Europe are less than conventional insecticides based on similarities in toxicity, exposure, and environmental fate which suggests that these types of pheromones pose minimal risk to human health and the environment (OECD, 2002; EPA, 2007). This assessment summarizes the range of toxicity data for the EGVM pheromone, as well as other acetate-based SCLP, and describes the potential environmental risk to terrestrial and aquatic organisms.

Figure 1. Isomate EGVM dispenser applied to vegetation.

Exposure Analysis

Terrestrial

Exposure to terrestrial organisms is expected to be minimal based on the previously described application method and fate of SCLP in the environment. In terrestrial environments, exposure can occur via dietary, dermal, or inhalation exposure. Dietary exposure can occur through the ingestion of the dispenser by mammals and birds; however, this type of exposure is not expected because dispensers are attached to trees and other objects, and would have to be physically removed and then ingested to receive any type of dose. Another route of dietary exposure that could occur is through the ingestion of food items in the area of treatment. Based on the volatility and other physical properties of the pheromone, significant residues are not expected on food items that mammals, birds, reptiles, and terrestrial insects might consume. The lack of residues has been documented for similar pheromones on different commodities (Spittler et al., 1992). Dermal exposure is also expected to be negligible because the pheromone is contained within a plastic tube.

Due to the volatile nature of insect pheromones, another route of potential exposure could be from inhalation. As a conservative method to quantify exposure, the maximum amount of pheromone available in 200 dispensers was assumed to be discharged instantaneously into a confined area that is 1 ha in size and 2-meters tall. Based on the maximum amount of active ingredient allowed on the label (38.36 g a.i./ac) results in a rate of 94.95 g a.i./ha after converting the rate from acres to hectares. Based on the above assumptions regarding the confined area of release, the maximum amount of pheromone available for inhalation would be 4.75 mg/m^3 if all the pheromone was released instantaneously. This estimate is highly conservative as the dispensers are designed to act as a barrier to allow passive diffusion of the pheromone into the surrounding environment over time (Brown et al., 1992; McDonough, 1993; Mayer and Mitchell, 1998). Diffusion rates for individual dispensers are typically in the low nanogram range but have been reported to be as high as 10 µg/dispenser/hour for other SCLP (Mayer and Mitchell,

1998). The maximum rate stated on the material safety data sheet for Isomate® EGVM is 35 µg/dispenser/hour (Pacific Biocontrol, 2010). This value is considered above what would be expected from the use of dispensers in this program as they are removed after 120 days, and smaller amounts of pheromone would need to be emitted to provide sufficient efficacy before replacing the dispensers. This value is still highly conservative because the pheromones would not be confined to the small closed area defined in this exposure assessment. In addition, these concentrations would not be constant as the release rate will decrease over time resulting in pulsed exposures (Mayer and Mitchell, 1998). Also, the above assumes that the pheromone would not degrade, which is a highly conservative assumption based on available data for SCLP. Over time, degradation and slower release rates from the dispensers would result in long-term concentrations well below the microgram range until the dispensers are replaced. Measured aerial pheromone concentrations in the field from placement of dispensers at recommended rates have been shown to range from the upper picogram to low nanogram level per cubic meter for *Pectinophora gossypiella* (Lepidoptera: Gelechiidae) and *Cydia pomonella* (Lepidoptera: Tortricidae), as well as other moth species (Suckling et al, 1999; Stelinksi et al., 2004).

Based on the above maximum exposure level for instantaneous discharge of all pheromone, species-specific inhalation doses were calculated for several surrogate species representing various inhalation rates for certain nontarget wild mammals, birds, and reptiles (table 1) (EPA, 1993; Birchard et al., 1984). In cases where there was a range of inhalation values, the highest value was used to maximize the dose.

Table 1. Species-specific Inhalation Dose of Isomate® EGVM for Nontarget Mammals, Birds, and Reptiles.

Species	Inhalation Rate (m^3/day)	Maximum Inhalation Dose (mg/m^3)	Species Specific Inhalation Dose (mg/day)
Deer mouse	0.025	4.75	0.12
Cottontail	0.63	4.75	2.99
Red fox	2.0	4.75	9.50
Belted kingfisher	0.094	4.75	0.47
Western gull	0.48	4.75	2.28
Bald eagle	1.43	4.75	6.79
Garter snake	4.2×10^{-5}	4.75	2.00×10^{-4}

Aquatic

SCLP exhibit chemical and physical properties that suggest that the probability of contamination of aquatic habitats would be very low. In addition, the proposed formulation and dispensers further reduces the possibility of contamination from runoff and drift. The primary method of off-site transport for most conventional pesticides is volatilization, drift, and runoff. The proposed method of application for pheromones in this program involves the use of dispensers that will be attached to trees and other structures by hand, thus allowing release of the pheromone over time. This method of application and type of formulation will eliminate drift and runoff as a mechanism of off-site transport. The chemical will volatilize into the air as this

is the natural mechanism of communication between female and male moths for reproduction purposes. Pheromone is expected to be dispersed primarily within the areas of treatment, although changes in weather conditions could result in some fraction of the pheromone moving outside of treated areas. Pheromones similar to the one proposed for use in this program report low solubility values in water. Most SCLP are considered insoluble in water with reported solubility values in the low part per billion range. The actual solubility value for the EGVM pheromone is unknown; however, it is expected to be low based on high volatility and solubility values that have been measured for other SCLP. In addition, the highly variable recoveries in all of the toxicity studies are consistent with a chemical that is poorly soluble in water. Formulation studies did not use a solvent carrier to increase solubility resulting in measured concentrations well outside the typical range of recoveries (80 to 120 percent) for products that are soluble in water. Additional solubility and stability studies with the light brown apple moth technical material and formulation suggest very low solubility based on analytical recovery data collected as part of the expanded ecological toxicity testing that was conducted on this pheromone and associated formulations (CDFG, 2009o). Based on nominal concentrations for the technical material and dispensers, reported analytical recoveries ranged from 16 to 420 percent.

Effects Analysis

Mammalian Toxicity

The reported acute toxicity values for SCLP, such as EGVM, show low oral, dermal, and inhalation toxicity. Available acute mammalian standardized toxicity data for approximately 10 structurally similar lepidopteran pheromones suggest that these types of compounds would be considered practically nontoxic with median lethal oral dose values ranging from greater than 5 grams per kilogram (g/kg) to greater than 34.6 g/kg. Acute dermal toxicity is also considered low with LD_{50} values ranging from greater than 2 g/kg to 20.25 g/kg based on study results from nine acetate based straight chain lepidopteran pheromones (Touhey, 1990; Inscoe and Ridgway, 1992). Inhalation hazards are also low based on results compiled from three studies that show that the LC_{50} values range from 3.3 to 33.2 milligrams per liter (mg/L) (Touhey, 1990; Inscoe and Ridgway, 1992; EPA, 1996; Weatherston and Stewart, 2002). Chronic toxicity data is limited for SCLP as the U.S. Environmental Protection Agency (EPA) waives these types of studies because of their low acute toxicity and the low potential for long-term exposure. Available subchronic and developmental mammalian toxicity studies have shown no mutagenic, carcinogenic, or developmental effects for all tested pheromones (Touhey, 1990; EPA, 1996). Daughtrey et al. (1990) dosed rats daily 5 days per week for 13 weeks with tridecyl acetate at doses ranging from 0.1 to 1.0 g/kg/day. The calculated no observable effect level was found to be 0.1 g/kg/day based on a slight increase in liver weight, which is consistent with long-term dosing of chemicals and is not specific to this group of pheromones.

Avian and Reptile Toxicity

EGVM pheromone toxicity to birds has not been characterized based on a review of the available literature. However, from acute avian studies that have been conducted using similar SCLP, toxicity to birds is considered to be low. Acute oral LD_{50} values for bobwhite quail are reported to be greater than 2 g/kg, while mallard values range from greater than 2 g/kg to greater than 10 g/kg (Weatherston and Stewart, 2002). A review of the literature demonstrates that toxicity

data for reptiles and SCLP is not available. EPA Office of Pesticide Programs (OPP) assumes in their ecological risk assessments that the sensitivity of birds is representative of the sensitivity of reptiles. There is uncertainty in this assumption which is addressed in the risk characterization section of this risk assessment.

Terrestrial Plant Toxicity

No direct toxicity to terrestrial plants is expected based on the mode of action of SCLP. Insect pheromones are specific to insects of the same species, and not expected to cause direct adverse impacts to terrestrial plants. Extensive use of insect pheromones in pest management activities has not reported negative impacts to crops or adjacent nontarget plants. The proposed formulation in this program will not result in direct application to plant surfaces because the dispensers are attached to vegetation.

Terrestrial Invertebrate Toxicity

Nontarget toxicity data for terrestrial invertebrates is limited to acute studies with the honey bee for other technical and formulated SCLP. Ingestion and contact exposures to a 0.1 or 1.0 percent concentration of the technical material and a microencapsulated formulation for the light brown apple moth, *Epiphyas postvittana*, did not result in any mortality or changes in food consumption at either concentration (Monheit et al., 2008). The lack of toxicity, or sublethal effects, at doses above those considered environmentally relevant suggests low acute toxicity. Toxicity to other terrestrial invertebrates is expected to be low based on the mode of action for how these types of compounds work, their species specificity, and their low toxicity to the honey bee and other taxa. The use of insect pheromones is designed to alter insect behavior; therefore, any nontarget terrestrial invertebrate impacts would be expected to be sublethal in nature. Sublethal impacts to other lepidopteran, such as butterflies, are not expected because of the specificity of the combination of pheromone that is present in the Isomate® EGVM dispenser. The production of mating pheromones is recognized as a process that is species-specific to attract potential males for reproduction purposes (Roelofs, 1995; Plettner, 2002; Howard and Bloomquist, 2005). This is supported by surveillance trap data for other SCLP that has been collected in California. Native moths collected in light brown apple moth sticky traps include six species (*Henricus umbrabasanus, Archips argyrospilus, Clepsis peritana, Clepsis fucana, Argyrotaenia franciscana, Platynota stultana*) of moths from the family Tortricidae, and one species (*Achyra occidentalis*) from the family Crambidae which, in some cases, is considered a subfamily within the Pyralidae family (CDFA, 2007). The collection of this group of moths is consistent with available pheromone data that suggests this group, as well as species from other closely related families, can be attracted to structurally similar pheromones (Pheronet, 2010). The collection of other nontarget insects in some of the surveillance traps may be more of a function of trap design, placement, and color than a response to the pheromone itself. Trap color, placement, and design, as well as other factors not related to the pheromone itself, have been shown to influence the collection of nontarget invertebrates (Mitchell et al., 1989; Gross and Carpenter, 1991; Clare et al., 2000). These types of impacts would not be expected with the use of dispensers because no trap is being used in the dispersion of the pheromone.

The specificity of pheromones to insects of the same species is not solely related to the unique chemical structure of a given insect pheromone. In the case of lepidopteran pheromones, the

chemical structure of each pheromone, and its blend, is a critical component in selecting for species specificity. The use of a primary component in lepidopteran pheromones to attract a specific group of Lepidoptera with the addition of other smaller components in the blend at a specific ratio has been recognized as a means of maximizing species specificity for reproduction (Ando et al. 2004; Baker, 1989; Rumbo et al., 1993; McDonough et al., 1993; Wyatt, 2003; Stelinski et al., 2007). Secondly, the species-specific nature of pheromones is evident in the receiving structure that elicits a response in the male insect (Roelofs, 1995). The specialized sensory system in male moths, as well as the physical structure of the antennae, further increase species specificity when compared to other insects, such as butterflies. Other lepidopterans, such as butterflies, have very different antennae structures, as well as reproductive behavior patterns, that further reduce the potential for moth SCLP to affect butterfly behavior and reproduction (Myers, 1972; Rutowski, 1991; Wyatt, 2003).

Aquatic Toxicity

Aquatic acute toxicity testing using other technical SCLP show that the range of toxicity values is greater than 100 mg/L to 540 mg/L for the bluegill sunfish, and greater than 100 mg/L to 270 mg/L for the rainbow trout (Weatherston and Minks, 1995). Other SCLP toxicity studies report acute EC_{50} values for aquatic invertebrates, such as the freshwater cladoceran, ranging from 1.20 to 6.80 mg/L (Weatherston and Minks, 1995; Rosa et al., 2006). Similar results for fish and aquatic invertebrate toxicity testing have been observed in several studies using the pheromone active ingredient for the light brown apple moth (Werner et al., 2007; CDFG, 2009a–g) (table 2). In all cases, toxicity values exceed solubility limits for SCLP and would not be expected to occur in the environment.

Table 2. Toxicity of Light Brown Apple Moth Technical Pheromone to Select Aquatic Species.

Test Organism	Exposure Duration	LC_{50}/EC_{50} (mg/L)	LOEC[2] (mg/L)	NOEC[3] (mg/L)
Ceriodaphnia dubia	96 hours	>262.4	>262.4	262.4
C. dubia[1]	7 days	120.19	100.0	50.0
Rainbow trout, *Oncorrynchus mykiss*	96 hours	>262.4	>262.4	262.4
Fathead minnow , *Pimephales promelas*	96 hours	>328.0	>328.0	328.0
Fathead minnow [1]	7 days	>200	>200	200
Bullfrog tadpoles, *Rana catesbiana*	96 hours	>114.8	>114.8	114.8
Green algae, *Selanastrum capricornutum*	96 hours	1.48	1.17	<1.17

[1] Response based on effects to reproduction
[2] Lowest observable effect concentration
[3] No observable effect concentration

Toxicity for the formulation proposed in this program is comparable to the available toxicity data for the technical material. Available aquatic toxicity data for the proposed formulation in this program suggests low acute and chronic toxicity based on available data (CDFG, 2011a–c; CDFG, 2010a–i) (table 3). All standardized studies listed in the below tables were conducted by the California Department of Fish and Game (CDFG). Aquatic exposures to the dispensers in

these studies were conducted by cutting the dispensers into 10 cm sections, adding a volume of water to obtain the desired concentration, and then shaking for 6 hours. This type of exposure is not expected given that the dispensers will be attached to vegetation and allowed to passively release pheromone over time.

Table 3. Toxicity of the Formulation Isomate® EGVM to Aquatic Species.

Test Organism	Exposure Duration	LC_{50}/EC_{50} (mg/L)	LOEC[2] (mg/L)	NOEC[3] (mg/L)
C. dubia	96 hours	0.789	0.9	<0.9
C. dubia[1]	7 days	4.795	1.5	0.75
Rainbow trout	96 hours	180.1	191.79	95.9
Fathead minnow	96 hours	121.8	47.95	23.98
Fathead minnow[1]	7 days	26.69	11.99	6.00
Hyallela azteca	96 hours	1.302	0.679	<0.679
Bullfrog tadpoles	96 hours	751	787	722
S. capricornutum	96 hours	0.385	0.622	0.094
Skeletonema costatum	96 hours	0.433	1.0	0.142

[1] Response based on effects to reproduction
[2] Lowest observable effect concentration
[3] No observable effect concentration.

Additional toxicity testing conducted using another dispenser formulation, Isomate® LBAM-Plus, shows similar low acute and chronic toxicity to aquatic invertebrates, fish, and algae, with response values above solubility limits for SCLP (CDFA, 2009h–n) (table 4). Apparent differences in toxicity between the two formulations are based on whether the toxicity values were based on expected or measured concentrations in determining the dose-response curves. Reported values for the LBAM formulation are based on expected concentrations, while those reported for the EGVM formulation were based on measured concentrations for most species. Low solubility for the SCLP results in very poor recoveries when using measured concentrations (CDFA, 2009o).

Table 4. Toxicity of the Formulation Isomate® LBAM-Plus to Select Aquatic Species.

Test Organism	Exposure Duration	LC_{50}/EC_{50} (mg/L)	LOEC[2] (mg/L)	NOEC[3] (mg/L)
C. dubia	96 hours	>187	>187	187
C. dubia[1]	7 days	>187	93.75	46.88
Rainbow trout	96 hours	>187	>187	187
Fathead minnow	96 hours	>187	>187	187
Fathead minnow[1]	7 days	>187	23.44	11.72
Bullfrog tadpoles	96 hours	>27.41	>27.41	27.41
S. capricornutum	96 hours	>137.2	12.84	3.14

[1] Response based on effects to reproduction
[2] Lowest observable effect concentration
[3] No observable effect concentration.

Risk Characterization

The risk of pheromones to aquatic and terrestrial organisms was determined by assessing the relationship between the effects and exposure data for each group of nontarget organisms. For terrestrial nontarget wildlife, a RQ value was determined for each group of organisms by taking the lowest available acute and chronic toxicity value, and comparing it to the estimated environmental concentration. This was done for terrestrial organisms to determine potential risk; however, aquatic risk was not evaluated using the same methods because the application and chemical properties of insect pheromones eliminate the potential for exposure.

Terrestrials—Mammals, Birds, and Reptiles

Risk from oral ingestion of pheromone dispensers to nontarget mammals and birds is not expected due to the lack of exposure and low toxicity values that have been determined for SCLP. Dispensers would have to be consumed for any sort of substantial exposure to occur which is unlikely as multiple dispensers would have to be physically removed and completely ingested to receive a large dose of pheromone.

Inhalation risk to nontarget terrestrial vertebrates is expected to be extremely low for a range of animals based on the available toxicity data and the highly conservative exposure scenario that was defined in the exposure analysis. Using the maximum exposure value and comparing it to the lowest inhalation toxicity value results in extremely low RQ values (table 5). These values are consistent with inhalation risks for other SCLP that have been estimated for other nontarget vertebrates (CDFA, 2010).

Table 5. Species-specific Inhalation Risk Quotient Values for Nontarget Mammals, Birds, and Reptiles Exposed to Isomate® EGVM.

Species	Species-specific Inhalation Dose (mg/day)	Inhalation Toxicity Value (mg/m^3)	Risk Quotient (RQ)
Deer mouse	0.12	3000	4.0×10^{-5}
Cottontail	2.99	3000	1.0×10^{-3}
Red fox	9.50	3000	3.2×10^{-3}
Belted kingfisher	0.47	3000	1.6×10^{-4}
Western gull	2.28	3000	7.6×10^{-4}
Bald eagle	6.79	3000	2.3×10^{-3}
Garter snake	2.00×10^{-4}	3000	6.7×10^{-6}

As a means of comparison, EPA–OPP establishes levels of concern (LOC) for non-listed and listed mammals, birds, and reptiles where RQ values below the LOC make the presumption of no risk (EPA, 2004). No LOC has been established for inhalation risk; however, for federally listed mammals, birds, and reptiles the acute dietary LOC is 0.1 which is a minimum of two orders of magnitude above the calculated RQ values that were estimated for this assessment. In reality, the risk would be much less because the pheromone will be discharged over a period of time, and would begin to degrade once it passes across the dispenser. In addition, the pheromone would not be confined to the small area that was defined in the exposure analysis, but would diffuse

into the surrounding environment. Uncertainty regarding the inhalation risk to birds and reptiles is largely based on the assumption that mammalian inhalation toxicity is similar for all groups. The low inhalation toxicity of SCLP to mammals with the extremely low risk that was calculated for animals based on overly conservative assumptions of exposure indicates that if birds and reptiles are more sensitive to inhalation of insect pheromones that they would have to be several orders of magnitude more sensitive for any risk to occur under the exposure assumptions in this risk assessment. From an exposure perspective this would seem unlikely as reptiles, in general, have lower inhalation rates when compared to mammals, and birds would have greater mobility thus reducing their exposure, when compared to mammals. In addition, actual concentrations measured in the field are in the picogram to nanogram per cubic meter range; therefore, acute and chronic toxicity would have to be $3 X10^{9}$ to $3 X 10^{12}$ greater than that which has been demonstrated in acute mammalian tests for any effects to occur to any terrestrial vertebrates.

Terrestrials—Plants and Invertebrates

Direct risk from the toxicity of pheromone dispensers to terrestrial plants is not expected as the insect pheromones are species-specific and not known to be toxic to plants. Indirect risks to plants from potential pollinator impacts would not be expected for most plants because the pheromones are only designed to impact the ability of male EGVM moths to find female moths of the same species.

Aquatic

The direct and indirect risk to aquatic organisms from the use of pheromone dispensers is expected to be extremely low based on the available toxicity data, the chemical fate of these types of pheromones, and the proposed use pattern. Toxicity values are above the solubility limits for similar types of pheromones, as well as the formulation proposed for EGVM. Therefore, any measured response is above concentrations that could occur in an aquatic environment. In addition, the method of application and low use rates for the EGVM pheromone further reduces the risk of offsite movement from drift and runoff that could result in any direct and indirect impacts to aquatic organisms. As previously mentioned, the pheromone will be applied by hand as a dispenser. Dispensers are placed on trees and other objects for a period of 120 days before they are removed. Label prohibitions regarding placement and disposal of dispensers in water will further reduce the possibility of contamination of aquatic sites. Application rates are also low using the proposed method of application. Dispensers are placed at a rate of approximately 200 per acre, with each dispenser containing 191.79 mg of pheromone per dispenser. Approximately 38.36 g/acre of pheromone will be released into the atmosphere over the lifespan of all 200 dispensers in the environment. Under a very unrealistic exposure scenario where all of the pheromone is discharged simultaneously, the resulting concentration in a 1-acre body of water 1-foot deep would be approximately 31.1 µg/L, assuming miscibility in water. Under this exposure scenario, the active ingredient from all dispensers ranging from greater than 3 acres to 190 acres would have to be discharged simultaneously to reach the lowest chronic NOEC values for algae and fish, respectively. The lack of risk to algae, aquatic invertebrates, and fish under an unrealistic exposure scenario would support the conclusion that direct or indirect risk, such as loss of food items or impacts to habitat, would not be expected for higher trophic aquatic organisms. These levels could not occur because all of the pheromone

would have to be discharged simultaneously from the dispenser, all dispensers would have to be placed into the water, and the pheromone would have to be soluble in water.

Spinosad

Spinosad is a bacterial-derived insecticide that contains two active ingredients, spinosyn A and D. The active ingredients are derivatives of the actinomycete, *Saccharopolyspora spinosa*, which occurs naturally in soil (figure 2).

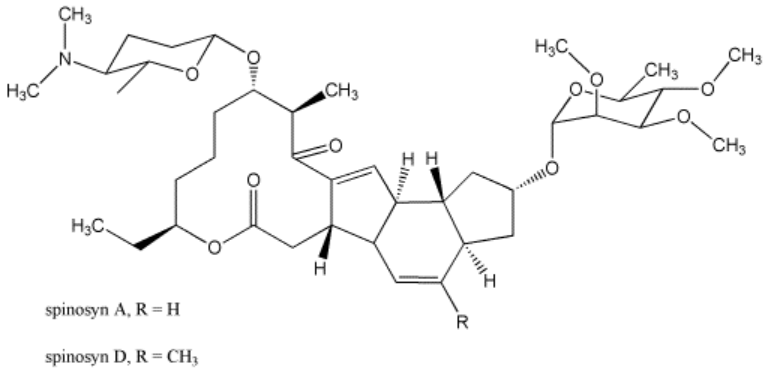

Figure 2. Chemical structure for spinosad.

The insecticide has been classified as a reduced risk insecticide by EPA due to its favorable human and environmental risk characterization and implementation into integrated pest management programs. Spinosad is a contact and primary ingestion insecticide with a mode of action that is unique to this class of compounds. The mode of action in insects is characterized by excitation of the nervous system, which eventually leads to paralysis with the effects consistent with the activation of the nicotinic acetylcholine receptors. The mode of action is unique when compared to nicotinic insecticides since the receptor site for each insecticide is different (Salgado, 1998; Thompson et al., 2000). The formulations proposed for use in this program are Entrust® and Success® which will be applied by ground equipment and used to treat EGVM larvae. Entrust® is for use in organic applications and is certified by OMRI.

Exposure Analysis

Spinosad persistence in the environment is variable in terrestrial and aquatic systems (table 6) (EPA, 1998). Spinosad is not sensitive to hydrolysis but breaks down rapidly in water in the presence of light with reported photolytic half-lives of less than 1 day. The rapid photolytic breakdown of spinosad in laboratory studies has also been confirmed in microcosm studies (Cleveland et al., 2002). Solubility of spinosad in water is pH dependent and is also dependent on the structurally similar active ingredients. Solubility for spinosyn A ranges from 290 to 16 mg/L with increasing pH, while the solubility for spinosyn D is much less but still pH-dependent with values ranging from 28.7 to 0.05 mg/L for pH values between five and nine (Cleveland et al., 2002).

Table 6. Reported Half-lives for Spinosad in Soil and Water.

Environmental Fate Parameter	Reported Half-life
Hydrolysis (Spinosyn A/D)	No degradation @ pH 5 and 7, pH 9 (200/259 days)
Aqueous Photolysis (Spinosyn A/D)	0.93/0.82 days @ pH 7
Soil Photolysis (Spinosyn A/D)	82/44 days
Aerobic Soil Metabolism (Spinosyn A/D)	9.0–17.3/14.5 days
Anaerobic Aquatic Metabolism (Spinosyn A/D)	161/ 205 days
Terrestrial Field Dissipation	0.3 to 0.5 days for Spinosyn A

Degradation of spinosyn A and D in soil is rapid under aerobic conditions suggesting spinosad is susceptible to microbial degradation (EPA, 1998a; Hale and Portwood, 1996). Spinosad also degrades quickly on plant surfaces with reported half-lives ranging from 2.0 to 11.7 days (CDPR, 2002; Sharma et al., 2008).

Spinosad is not considered mobile based on the available soil adsorption (K_{oc}) studies that have been conducted on a range of soil types. Values range from 884 to 145,350, with the lowest value occurring in a loamy sand with 1.1 percent organic matter and a cation exchange capacity (cec) of 1.9, while the highest value is for a silt loam soil with 0.4 percent organic matter and a cec of 12.0 (CDPR, 2002).

Spinosad is not considered to be volatile based on the vapor pressure for both active ingredients, with values of 2.4×10^{-10} mm Hg for spinosyn A and 1.6×10^{-10} mm Hg for spinosyn D (Cleveland et al., 2002). Chemicals with vapor pressure values less that 1×10^{-6} are considered nonvolatile (CDPR, 2002).

Expected aquatic and terrestrial residues from program applications of spinosad can be estimated using various environmental fate models. While off-site movement from spinosad is expected to be minimized by the application method and environmental fate for the product, residues were estimated to determine appropriate mitigation, such as buffer zones from listed aquatic and terrestrial species and their habitat. Application rates for this program were obtained from the Entrust® label, which is a formulation of spinosad that has been certified by OMRI for a wide variety of uses. This product contains 80 percent spinosyn A and D by weight and can be applied at a range of application rates, depending on the use and pest. Applications to control leafrollers, such as EGVM, can vary; however, the maximum rate proposed for the EGVM program is 3 oz Entrust®/acre. The maximum rate for Entrust® applications was used to estimate terrestrial and aquatic residue levels. The proposed use pattern for spinosad in this program is to make ground treatments to vegetation in areas within a 500-meter radius of a trap detection, or in nursery applications in areas under quarantine.

The primary model that was used in this assessment to determine off-site residues is the AgDrift model. AgDrift is a pesticide drift deposition model that provides the user with the ability to provide application-specific information as input to determine application efficiency and off-site drift residues. AgDrift is based on AgDisp which is a model that was developed by the U.S. Department of Agriculture's Forest Service. AgDrift has become a regulatory tool for EPA–OPP in the registration of pesticides (Hewitt et al., 2002; Teske and Curbishly, 2003). Both models

have a tiered approach that allows the user to choose default values, or provide more specific data based on available information. Both models have been validated under various application scenarios (Duan et al., 1992a; Duan et al., 1992b; Bird et al., 2002; Teske et al., 2000).

Based on the available options in AgDrift for modeling off-site transport of pesticides, the tier one high boom (50 in) broadcast ground application method was selected. This application method is conservative because applications are broadcast applied to bare ground. In the EGVM program, applications would be made using backpack sprayers and other ground equipment in noncommercial areas and nurseries resulting in less drift as they would cover smaller areas and applications would be directed to plant material. Due to the assumptions in the model, this provides a very conservative estimate of potential off-site drift for the proposed use pattern. This conservative approach was used to account for additional pesticides that may be introduced through runoff which is not accounted for in the AgDrift model. The median droplet size assumed in this scenario is 341 µm based on an American Society of Agricultural Engineers (ASAE) droplet size distribution class of fine to medium/coarse. A larger droplet size distribution can be used which would further reduce drift from the site of application. Wind direction was assumed to be at -90^{o} directly towards the sensitive habitat for the entire length of every row and blowing at a sustained speed of 7 mph (figure 3).

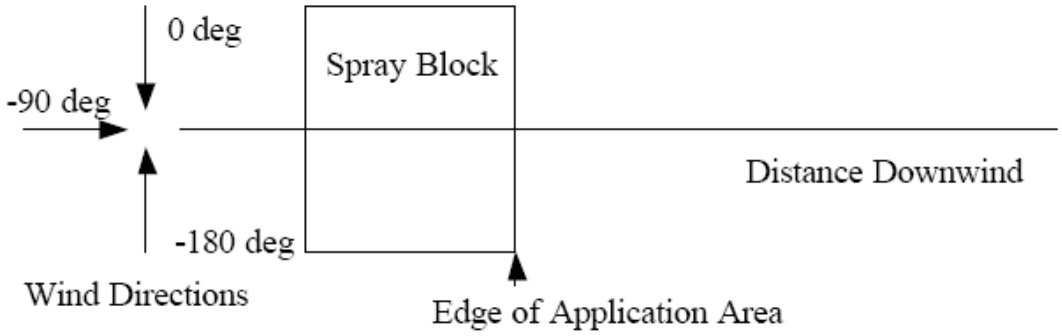

Figure 3. Wind direction relative to the spray block and the distance downwind.

The above assumptions in the drift modeling were also used for the other three insecticides that are discussed later in this risk assessment. Use rates selected for spinosad and the other insecticides were based on the maximum labeled rates and application frequency for grapes. Rates of treatment and frequency may be lower, depending on the application and use pattern; however, the intent of the exposure modeling was to generate worst-case residue values that could be compared to available toxicity data. Residues estimated from AgDrift represent 90th percentile residues that would be expected based on the method of application.

Terrestrial

Based on the maximum use rate proposed for spinosad in grapes (0.125 lb/ac), and the above assumptions, point estimates of drift to nontarget terrestrial environments were calculated at the edge of the spray block and at a range of distances downwind of the spray block into sensitive

habitat. A point estimate was used to generate a more conservative drift value as opposed to integrating the deposition over a given area downwind of the spray block. The point estimate of residues at 25 feet away from the spray block decreased approximately 98 percent from the edge of the spray block using a high boom application method (table 7). Drift declines asymptotically until the maximum allowable buffer zone in the model is selected. The decline in drift is large from edge of field to the 25-foot buffer when compared to the additional buffer zone distances. The rapid deposition observed in this modeling scenario is typical of what is observed in other drift modeling applications, as well as in field applications. The larger droplets are removed from the distribution at the shorter distances and then, as you move further downwind, you are selecting from a smaller distribution of spray droplets for deposition.

Table 7. AgDrift Estimate of Drift and the Reduction of Spinosad Residues from Applications to Sensitive Terrestrial Habitats.

Chemical	Buffer Zone (ft)	Fraction of Applied	Percent Reduction in Residues
Spinosad	0	–	–
	25	0.0208	97.92
	50	0.0119	98.81
	75	0.0087	99.13
	100	0.007	99.30

Exposure levels on terrestrial forage items were calculated using the Terrestrial Residue Exposure Model (T-REX) (EPA, 2008a). T-REX provides an updated version of the Fletcher residue model that was originally based on the Kenaga nomogram used by EPA–OPP in their risk assessment process for pesticide registration (Hoeger and Kenaga, 1972). T-REX allows the user to input variables, such as use, application rate/type, percent active ingredient, foliar dissipation half-life, application interval, and number of applications to calculate exposure concentrations on a variety of food items (table 8). For foliar sprays, the estimates of exposure are based on the original Kenaga nomogram using field collected residue data for several pesticide classes to calculate residue levels for a variety of food items. The model was updated by Fletcher to account for any potential differences in new chemistry classes that had been developed after Kenaga (Fletcher et al., 1994). Based on over 200 residue studies, the model was shown to provide an accurate representation of residues for certain food items.

Table 8. Input Parameters for T-REX Modeling.

Parameters	Spinosad
Application rate (lb ai/ac)	0.125
Half-life (days)	17.3
Number of Applications	2
Application Interval (days)	14

ai = active ingredient

Exposure concentrations for terrestrial receptors can be based on mg/kg diet or mg/kg body weight. Acute exposure concentrations based on the mean estimate of mg/kg diet for the highest

use rate of spinosad are listed below (table 9). These concentrations represent those residue levels that would be expected from a direct application to the listed food items.

Table 9. Expected Upper Bound Spinosad Residues (ppm) on Terrestrial Food Items Using T-REX.

Food Items	Spinosad Residues (ppm)
Short grass	30.00
Tall grass	13.75
Broadleaf plants/small insects	16.88
Fruits/pods/seeds/large insects	1.88

The values listed above can be used to calculate exposure concentrations based on mg/kg body weight and adjusted for different receptor classes using known ingestion rates. Mammal body weights, ingestion rates, and percent of body weight consumed values are listed below for a representative herbivore, insectivore, and granivore mammal (table 10).

Table 10. Adjusted Mammal Parameters for Different Class and Body Size.

Mammalian Class	Body Weight (g)	Ingestion (dry) (g bwt/day)	Ingestion (wet) (g/day)	% body wt consumed	Food Intake (kg-diet/day)
Herbivores/ Insectivores	15	3	14	95	1.43E-02
	35	5	23	66	2.31E-02
	1000	31	153	15	1.53E-01
Granivores	15	3	3	21	3.18E-03
	35	5	5	15	5.13E-03
	1000	31	34	3	3.40E-02

Using these values, estimated environmental concentrations (EEC) can be calculated based on the dose that each type of mammal would receive from different food items (table 11). This value can then be compared to the adjusted LD_{50} and NOEL values to determine if exposure levels exceed effect thresholds.

Table 11. Dose-based Spinosad Estimated Environmental Concentrations for Mammals.

Dose-based EEC (mg/kg-bw)	Mammalian Classes and Body Weight					
	Herbivores/Insectivores			Granivores		
	15 g	35 g	1000 g	15 g	35 g	1,000 g
Short grass	28.60	19.77	4.58			
Tall grass	13.11	9.06	2.10			
Broadleaf plants/small insects	16.09	11.12	2.58			
Fruits/pods/seeds/large insects	1.79	1.24	0.29	0.40	0.27	0.06

Doses for birds can also be calculated based on body weight and consumption rates for a range of bird sizes (table 12). For this assessment, birds ranging in size from 20 to 1000 g were used to calculate the amount of food that could be consumed in a given day.

Table 12. Adjusted Avian Parameters for Different Class and Body Size.

Avian Class	Body Weight (g)	Ingestion (dry) (g bw/day)	Ingestion (wet) (g/day)	% Body Weight Consumed	(kg-diet/day)
Small	20	5	23	114	2.28E-02
Mid	100	13	65	65	6.49E-02
Large	1000	58	291	29	2.91E-01

Dose-based EECs can also be made for different avian groups using the residues from table 6 so that doses based on body weight and feeding preference can be compared to the adjusted toxicity effects endpoint (table 13). These comparisons are discussed in the risk characterization section of this risk assessment.

Table 13. Estimated Spinosad Environmental Concentrations for Several Avian Classes.

Dose-based EEC (mg/kg-bw)	Avian Classes and Body Weights		
	small 20 g	mid 100 g	large 1000 g
Short grass	34.17	19.48	8.72
Tall grass	15.66	8.93	4.00
Broadleaf plants/small insects	19.22	10.96	4.91
Fruits/pods/large insects	2.14	1.22	0.55

Aquatic

The same application scenario previously described was used to estimate potential aquatic residues from program applications of spinosad. A range of aquatic habitats were modeled to determine potential residues. An aquatic habitat 10 feet wide by 6 inches deep was modeled as the most sensitive habitat, while a pond 1 acre in size and 6.56 feet deep was used as the larger habitat. The calculated residues do not account for any degradation of spinosad that would occur, nor does it account for the contribution from runoff that could occur in the event of a rainfall event. The AgDrift model only estimates potential drift from pesticide applications.

Based on the previously described application scenarios and the size of the aquatic habitat, the instantaneous edge of field aquatic residue was estimated to be 17.6 parts per billion (ppb) (table 14).

Table 14. Estimated Spinosad Residues at Edge of Field and Various Buffer Zones into a Shallow Closed Aquatic Habitat.

Chemical	Buffer zone (ft)	Aquatic Residues (µg/L)	Percent Reduction in Residues
Spinosad	0	14.8	-
	25	1.7	88.5
	50	1.0	93.2
	75	0.8	94.6
	100	0.6	96.0

Drift is considered the primary means of off-site movement for spinosad under this use pattern. Spinosad exhibits rapid dissipation in terrestrial environments with half-lives ranging from 0.3 to 0.5 days. Significant rainfall events immediately after application would have to occur to remove spinosad from foliage or soil to provide any residues that would be additive to the contribution from drift. In the case that a rainfall event were to occur, spinosad has a high binding affinity for soil which would reduce its potential for off-site transport via runoff in the dissolved fraction. The method of application proposed in this program also reduces the potential for spinosad to be applied to soil and removed in a rainfall event. Finally, any buffers and other best management practices related to application activities adjacent to aquatic habitats will further reduce the potential contribution from runoff during program applications.

Effects Analysis

Mammalian Toxicity

Technical spinosad has low acute toxicity to mammals with LD_{50} values ranging from 3,837 mg/kg to greater than 5,000 mg/kg for the rat and mouse (EPA, 1998a; Cleveland et al., 2001). Spinosad also has low dermal and inhalation toxicity with a dermal LD_{50} in the rat of greater than 2,000 mg/kg, and a rabbit inhalation LC_{50} value greater than 5.18 mg/L in the rabbit. Comparative toxicity data between the technical material and formulated spinosad demonstrate equivalent low acute toxicity through oral, dermal, and inhalation routes (EPA, 1998a). Several subacute, as well as longer term studies, are also available and suggest low to moderate chronic toxicity, depending on the endpoint (EPA, 1998a; EPA, 2006; Yano et al., 2002; Stebbins et al., 2002; Breslin et al., 2000) (table 15).

In addition to the above studies, spinosad is not considered to be mutagenic or carcinogenic (EPA, 1998a).

Using the lowest reported acute LD_{50} value and the lowest subchronic rat NOEL, adjusted LD_{50} and NOEL values were calculated for different size mammals (table 16). The ranges of different body weights and consumption rates were selected to represent mammals, such as the shrew that consumes a large percentage of their body weight and may receive higher exposure, as well as larger mammals (1 kg) that may consume less when compared to the standard laboratory rat, which is used in several of the mammalian toxicity studies. The rat subchronic value was used as the average weight could be approximated more easily than in the dog study where animal

Table 15. Subchronic and Chronic Mammalian Toxicity Values for Spinosad

Toxicity Test/ Test Species	NOEL (mg/kg/day)
Subchronic 13-week/Rat	8.6 males / 10.6 females
Subchronic 13-week/Rat	24
Subchronic 13-week/Mouse	7.5
Subchronic 13-week/Dog	4.85 males / 5.38 females
Chronic 1-yr/Dog	2.68 males / 2.72 females
Neurotoxicity 1-yr/Rat	50.3 males / 63.8 females
Chronic/Carcinogenicity 2-yr/Rat	2.40 males / 3.00 females
Carcinogenicity 18-month/Mouse	11.4 males / 13.8 females
Developmental Toxicity/Rat	50 maternal/ 200 developmental
Developmental Toxicity/Rabbit	10 maternal / 50 developmental
Reproductive Toxicity/Rat	10 developmental

Table 16. Adjusted LD_{50} and NOEL Values for Select Mammals.

Mammalian Class	Body Weight (g)	% Body Weight Consumed	Adjusted LD_{50}	Adjusted NOEL
	15	95	8433.08	16.48
Herbivores/ Insectivores	35	66	6823.26	13.34
	1,000	15	2951.27	5.77
	15	21	8433.08	16.48
Granivores	35	15	6823.26	13.34
	1,000	3	2951.27	5.77

weights were not reported. The lowest subchronic value was selected due to the short environmental half-life of spinosad. Effects from daily exposure over a 13-week period are not expected but provide a conservative estimate of effects. These values are also lower than any of the developmental and reproductive NOEL values that have been determined in previous studies.

Avian and Reptile Toxicity

Spinosad acute and chronic toxicity to birds is low for both surrogate species that have been tested (table 17). Acute LD_{50} and LC_{50} for the mallard and bobwhite quail are greater than the highest concentration tested, 1,333 mg/kg and 5,156 parts per million (ppm), respectively (EPA, 2010). Chronic toxicity is also low with reproduction NOEC values of 500 ppm for the mallard and 550 ppm for the bobwhite quail in 20-week exposure studies.

Table 17. Acute and Chronic Avian Toxicity of Spinosad.

Test Species/ Duration	LD_{50}/LC_{50} (mg/kg)	NOEL/LOEL (mg/kg)
Bobwhite quail, *Colinus virginianus* LD_{50}	>1333	500/NR
Bobwhite quail LC_{50}	>5156	656/NR
Bobwhite quail chronic reproduction	NR	550/1100
Mallard, *Anas platyrhynchos* LD_{50}	>1333	1333/NR
Mallard LC_{50}	>5156	302/NR
Mallard chronic reproduction	NR	500/1100

NR = Not reported

The lowest acute NOEL value (500 mg/kg) was used to estimate an adjusted sublethal toxicity value for birds of different sizes and feeding rates (table 18). Based on the adjusted body weight for different avian size classes and their percentage of body weight consumed, adjusted LD_{50} values ranged from approximately 360 to 648 mg/kg.

Table 18. Adjusted Acute Toxicity Values for Different Sized Avian Receptors.

Avian Class	Body Weight (g)	% Body Weight Consumed	Adjusted NOEL
Small	20	114	360.21
Mid	100	65	458.57
Large	1000	29	647.75

No reptile toxicity data appears to be available for spinosad. EPA–OPP uses the effects data for birds to represent sensitivity to reptiles. There is uncertainty in this assumption; however, based on the low toxicity of spinosad to birds and mammals, as well as aquatic vertebrates, toxicity to reptiles would also be expected to be low.

Terrestrial Invertebrate Toxicity

Toxicity to terrestrial invertebrates is variable based on the available toxicity data for pests, pollinators, and biocontrol agents. Honey bees appear to be one of the more sensitive terrestrial invertebrates to spinosad, with 48-hour contact LD_{50} values ranging from 0.0029 to 0.078 µg ai/bee and a reported NOEC of 0.0016 µg ai/bee (0.016 µg/g) (Mayes et al., 2003). Toxicity to honey bees is similar to other native bees with reported contact LD_{50} values of 0.058, 0.065, and 0.078 µg ai/bee for the alfalfa leafcutter bee (*Megachile rotundata*), alkali bee (*Nomia melanderi*) and honey bee, respectively (Mayer et al., 2001). Contact toxicity to spinosad decreases rapidly after applications are allowed to dry. Laboratory, greenhouse, and field studies have demonstrated that spinosad is nontoxic to bees 3 hours after application (Mayes et al., 2003). Studies using honey bees and bumblebees exposed to spinosad residues on alfalfa, strawberries, almonds, citrus, and kiwifruit have documented a lack of impacts to pollinators when applications are made when bees are not active, and after residues have weathered. The results of these studies are reflected on the label language for all spinosad products stating that

applications should not be made to blooming, pollen-shedding, or nectar-producing plants during a time period of 3 hours when bees are active.

Toxicity to other nontarget insects ranges from 3.3 to greater than 200 mg/L based on reported LC_{50} values (Thompson et al., 2000; Williams et al., 2003; Penagos et al., 2005; Miles and Eelen, 2006; Semiz et al., 2006). Within Lepidoptera, sensitivity can vary with effective treatment rates ranging from 25 to 360 g/ha (Thompson et al., 2000). Lepidoptera appear to be less sensitive to spinosad compared to pollinators, such as honey bees and bumblebees. For example, contact toxicity of fourth instar *Spodoptera littoralis* larvae to spinosad is reported as 4.74 µg/g, which is lower than the 0.029 µg/g reported for the honey bee (Pineda et al., 2006). Dietary spinosad LC_{50} values for *S. littoralis* range from 0.5 to 2.98 ppm.

Based on field-collected data, there were no effects on abundance and diversity of Lepidoptera, Coleoptera, or Hymenoptera when sampled using malaise traps 2 and 6 days after spinosad treatment for emerald ash borer (USDA–APHIS, 2007). Aerial applications were made to several plots ranging in size from 8 to 20 acres at a rate (0.23 lb ai/ac) which is above the proposed treatment area and use rate proposed in the EGVM eradication program.

Terrestrial Plant Toxicity

No terrestrial phytotoxicity has been noted using spinosad at rates up to 0.18 lb ai/ac (EPA, 1998a).

Aquatic Toxicity

Spinosad has moderate toxicity to fish based on the available toxicity data, with acute toxicity values ranging from 4.99 to 30 mg/L in 96-hour exposures (table 19).

Table 19. Spinosad Aquatic Toxicity Values for Aquatic Vertebrates.

Test Species/Duration	LC_{50}/EC_{50} (mg/L)	NOEC/LOEC (mg/L)
Acute Tests		
96-hour LC_{50} Carp *Cyprinus carpio*	4.99	NR
96-hour LC_{50} Bluegill Sunfish *Lepomis macrochirus*	5.9	2.10/NR
96-hour LC_{50} Rainbow Trout	30	5.2/NR
96-hour LC_{50} Sheepshead Minnow *Cyprinodon variegates*	7.87	1.8/NR
Subchronic Tests		
Rainbow Trout ELS*	NR	0.498/0.962
Rainbow Trout 21-d	NR	1.2/2.1
Sheepshead 35-d ELS	NR	1.15/2.38

*ELS = Early life stage study; NR = Not reported

A literature review revealed no apparent toxicity data for spinosad on amphibians. EPA–OPP uses fish toxicity data to represent the sensitivity of amphibians which provides uncertainty due to potential differences in sensitivities, and differences in exposure pathways between fish and

adult amphibians. However, due to the lack of amphibian-related data for spinosad, fish effects data will be used as a surrogate and discussed in relation to risk in the below aquatic risk characterization section.

Based on the available aquatic toxicity profile, spinosad has variable toxicity to aquatic invertebrates (table 20) (EPA, 1998a; Stark and Banks, 2001; Cleveland et al., 2001). Longer term studies show that under continuous exposure for 21 days, spinosad has a greater effect on the freshwater cladoceran, *Daphnia magna*. The same study using pulse doses within a short period of time demonstrates the rapid breakdown of spinosad with an approximate tenfold decrease in toxicity. Stark and Vargas (2003) demonstrated demographic effects to *D. pulex* populations exposed to formulated spinosad at 8 µg/L and greater in 60- to 70-day exposures. Test chambers were renewed every other day for the duration of the study. In a long-term sediment study, the midge *Chironomus riparius* was shown to have lower chronic sensitivity when compared to *D. magna* tested under continuous exposure conditions.

Table 20. Spinosad Aquatic Toxicity Values for Aquatic Invertebrates.

Test Duration/Species	LC_{50}/EC_{50} (mg/L)	NOEC/LOEC (mg/L)
Acute Tests		
48-hour EC_{50} *Daphnia magna*	14.0	0.45/NR
96-hour LC_{50} Grass Shrimp	>9.76	1.66/NR
96-hour LC_{50} Eastern Oyster	0.30	0.14/NR
24-hour LC_{50} *Aedes aegypti*	0.025	NR
24-hour LC_{50} *A. aegypti* (4^{th} instar)	0.160	NR
72-hour LC_{50} *A. aegypti* (Adults)	0.460	NR
Chronic Tests		
21-day LC_{50} *D. magna* (continuous)	>0.006	NR
21-day LC_{50} *D. magna* (5-d pulse)	>0.057	NR
28-day Mysid Life Cycle	NR	0.084/0.173
25-day LC_{50} *Chironomus riparius*	> 0.003	1.6
21-day *D. magna* (continuous)	NR	0.617/1.2 (µg/L)
21-day *D. magna* (5-d pulse)	NR	1.6/3.2 (µg/L)
25-day *C. riparius*	NR	84.2/173 (µg/L)

NR = Not reported

Mosquito species, such as *Culex pipiens, Aedes aegypti*, and *A. albimanus* appear to be the most sensitive aquatic invertebate taxa to spinosad, while the cladoceran *D. magna* was the least sensitive with a 48-hour EC_{50} of 14 mg/L (EPA, 1998a; Bond et al., 2004). Mosquito sensitivity was comparable between technical and formulated spinosad based on the available data (Bond et al., 2004; Cetin et al., 2005; Stevens et al., 2005; Ayesa et al., 2006). Acute aquatic toxicity studies conducted testing the Success® formulation of spinosad demonstrated comparably higher toxicity to *D. magna* when compared to the available value for the technical material, but lower toxicity to fish (Deardorff and Stark, 2009) (table 21). The material safety data sheet states that 22.8 percent of the formulated material is the active ingredient, while the remaining balance is

Table 21. Formulation Toxicity of Spinosad to Aquatic Organisms.

Test Duration/Species	LC_{50}/EC_{50} (mg/L)	Formulation
48-hour EC_{50} *D. magna*	0.0048	Success®
48-hour EC_{50} *D. pulex*	0.1290	Success®
48-hour EC_{50} *C. dubia*	0.0018	Success®
96-hour LC_{50} Coho salmon *O. kisutch*	>500	Success®
24-hour LC_{50} *C. tepperi* (4th instar)	0.029	Success®
24-hour LC_{50} *Culex pipiens* (3rd instar)	0.027	Conserve®
24-hour LC_{50} *C. pipiens* (4th instar)	0.111	Conserve®
24-hour LC_{50} *C. pipiens*	0.002–0.0022	Conserve®/Tracer®
72-hour LC_{50} *A. albimanus*	0.024	NR

unknown with the exception of one ingredient—1,2 propanediol (propylene glycol)—which composes an unreported percentage of the other materials (Dow, 2004). Propylene glycol toxicity to *D. magna* is low with reported EC_{50} and NOEC values greater than 1000 mg/L (EPA, 2010). The other formulation proposed for use in this program, Entrust®, contains 80 percent active ingredient with an additional 3.4 percent composing porcelain clay, while the remaining 16.6 percent is composed of other ingredients according to the material safety data sheet (Dow, 2007). All values fall within the range of acute and chronic effect values that have been reported for aquatic invertebrate exposure to the technical material.

Toxicity to aquatic plants, such as diatoms and algae, range from 0.107 mg/L for the freshwater diatom, *Navicula pelliculosa*, to greater than 105.5 mg/L for green algae based on 5-day exposures (EPA, 2010). Toxicity to aquatic macrophytes is based on a 14-day EC_{50} value for duckweed, *Lemna gibba*, which was reported as 10.6 mg/L.

Available acute and chronic aquatic toxicity data for metabolites of spinosyn A and D demonstrate that metabolites are less toxic than the parent material (table 22) (EPA, 2010). The spinosyn A metabolite has a sublethal toxicity value that is more than 160 times below the reported spinosad NOEC for *D. magna*. The same is true when comparing chronic toxicity values for *D. magna* between the parent and metabolite. Based on NOEC values from the 21-day studies, the spinosyn A metabolite is more than 15,000 times less toxic than the parent. Similar reductions in toxicity are also observed when comparing spinosad aquatic invertebrate toxicity to the primary metabolite for spinosyn D.

Table 22. Spinosyn Metabolite Aquatic Toxicity Values for Aquatic Biota.

Test Duration/ Species	LC_{50}/EC_{50} (mg/L)	NOEC/LOEC (mg/L)
Spinosyn A metabolite		
48-hour EC_{50} *D. magna*	> 197.0	74.7/NR
21-day *D. magna* reproduction	NR	9.32/>9.32
28-day *C. riparius*	>0.073	0.073/NR
96-hour *Navicula pelliculosa*	31.0	17.2/NR
Spinosyn D metabolite		
48-hour EC_{50} *D. magna*	66.8	46.4/NR
21-day *D. magna* reproduction	NR	4.85/9.32
28-day *C. riparius*	>0.039	0.039
96-hour *N. pelliculosa*	19.0	14.2/NR
120-hour *N. pelliculosa*	0.22	0.17/NR

NR = Not reported

Risk Characterization

Terrestrial (Mammals, Birds, and Reptiles)

Direct acute and chronic risk to mammals was determined by comparing the weight adjusted estimated environmental concentrations to the adjusted acute LD_{50} and NOEL values (table 23). All acute RQ values were less than 0.01, suggesting minimal acute risk to mammals. As a point of reference, EPA–OPP establishes levels of concern for different taxa; when those levels are exceeded, there is a presumption of risk. For mammals, the level of concern for acute risk to endangered mammals is 0.1. Acute RQ are greater than an order of magnitude above the EPA–OPP level of concern for endangered mammals. Chronic RQs for the 15 and 35 g mammals that feed exclusively on short grass or small insects were slightly above one suggesting possible chronic risk; however, the estimates of risk are based on very conservative assumptions regarding exposure and the effects. Actual risk would be much lower using a more appropriate chronic endpoint. The use of a 13-week NOEL in this study is very conservative as spinosad residues in the field would only persist for few days, based on laboratory and field studies. In addition, the estimates of exposure were upper bound estimates based on maximum application rates using the longest laboratory derived aerobic soil metabolism half-life value (table 8). Using the terrestrial field dissipation half-life value of 0.5 days provides a more realistic degradation profile, and would reduce the chronic RQ below one. This estimate of risk also assumes that mammals would only be feeding on contaminated prey which is unlikely to occur because spinosad applications will only occur in small areas, and would be less than the foraging range for most mammals.

Table 23. Dose-based RQ Values for Multiple Mammal Classes.

Dose-based RQs (Dose-based EEC/LD50 or NOEL)	15 g mammal		35 g mammal		1,000 g mammal	
	Acute	Chronic	Acute	Chronic	Acute	Chronic
Short grass	<0.01	1.74	<0.01	1.48	<0.01	0.79
Tall grass	<0.01	0.80	<0.01	0.68	<0.01	0.36
Broadleaf plants/small insects	<0.01	0.98	<0.01	0.83	<0.01	0.45
Fruits/pods/large insects	<0.01	0.11	<0.01	0.09	<0.01	0.05
Seeds (granivore)	<0.01	0.02	<0.01	0.02	<0.01	0.01

Acute direct risk to avian species is also considered low, with exposure levels well below the sublethal dose-based NOEL that was selected in the effects analysis. The RQ values ranged from less than 0.01 for larger birds feeding exclusively on insects and seeds, to 0.11 for small birds consuming short grass (table 24).

Table 24. Dose-based Risk Quotient Values for Multiple Avian Classes.

Dose-based RQs (Dose-based EEC/NOEL)	Avian Acute RQs		
	20 g	100 g	1,000 g
Short grass	0.09	0.04	0.01
Tall grass	0.04	0.02	0.01
Broadleaf plants/small insects	0.05	0.02	0.01
Fruits/pods/large insects	0.01	<0.01	<0.01

Chronic risk to birds was estimated by taking the highest spinosad residue level for all food items (short grass—36.00 ppm) and comparing that value to the lowest reproductive bird NOEC value (500 ppm). The RQ value was estimated to be 0.07 suggesting minimal chronic risk to birds. Chronic risk is based on the assumption that spinosad is persistent, which is not the case based on laboratory and field degradation/dissipation studies. Dose-based RQ values could not be calculated because the ingestion rates and body weights of birds used in the study were not given in the summary information provided by EPA. The acute and chronic risks established for birds are also assumed to apply to reptiles. There is uncertainty in this assumption; however, reptiles would have to be greater than two orders of magnitude more sensitive to spinosad than birds, based on conservative assumptions regarding exposure to demonstrate potential direct risks. This would seem unlikely based on the comparatively lower ingestion and metabolism rates for reptiles when compared to birds.

Indirect risk to mammals, birds, and reptiles could occur from the loss of terrestrial invertebrate prey for those terrestrial vertebrates that depend on terrestrial insects. Impacts to some terrestrial invertebrates are expected for vegetation that is directly treated with spinosad; however, not all terrestrial invertebrates will be impacted based on the range of sensitivities for invertebrates, the lack of control of all life stages, and the low residual contact toxicity of spinosad 3 hours after application. In addition, treatments are not broadcast applied over a large area but are focused on

host plants within a 500-meter radius of an EGVM detection or in a nursery setting. Adjacent shrubs and trees would continue to hold insect populations while ground-dwelling insects would also be available as impacts to that group of terrestrial invertebrates would be less.

For those terrestrial vertebrates that depend on aquatic invertebrates or fish, no indirect impacts would be expected based on the characterization of risk to aquatic biota (which is discussed in the next section). Additionally, there is also a low probability of risk from the ingestion of potentially contaminated aquatic prey as spinosad has a bioconcentration factor of 28 to 152 suggesting it does not bioconcentrate in fish (EPA, 1998a).

Terrestrial (Plants and Invertebrates)

Based on the known phytotoxicity of spinosad and its proposed use in the EGVM program, no direct risk is expected for terrestrial plants. Indirect risk through the loss of pollinators is expected to be minimal and localized based on the use pattern and fate of spinosad. In addition, precautionary label language will further reduce potential risk to pollinators. Current label language states "Do not apply this pesticide to blooming, pollen-shedding or nectar producing plants if bees may forage on the plants during this time period." The time period referred to on the label is a 3-hour time period after application when the residual toxicity to bees is high. Application buffer zones from listed plants, as well as listed terrestrial invertebrate habitat, will also reduce exposure and risk. Risk to other terrestrial invertebrates from contact with spinosad is expected to be comparable to those described for the honey bee. The honey bee appears to be the more sensitive insect when comparing toxicity values to other insects; therefore, risk would be expected to be equal or less than those calculated for the honey bee (Thompson et. al., 2000). As mentioned previously, applications will not be broadcast applied but will be directed to plants within a 500-meter radius of EGVM trap detections or to plants in production nurseries. The smaller areas of treatment, direct application to plants, and short residual toxicity will facilitate a faster re-introduction of any potentially impacted terrestrial invertebrates into the treated area. In addition, the implementation of best management practices, such as using larger droplets and avoiding applications during high wind speeds, will also minimize impacts to off-site terrestrial invertebrates.

Aquatic

Acute and chronic direct risk to fish and amphibians was determined by comparing the range of effects data to the range of exposure values. Aquatic residues were at least an order of magnitude lower than the range of available toxicity data suggesting spinosad would not pose a risk to fish populations (figure 4). Acute and chronic risk would actually be lower as the toxicity values are based on constant exposures using flow-through studies, whereas in the field spinosad would break down quickly in aquatic systems to its less toxic metabolite, or partition to sediment reducing bioavailability.

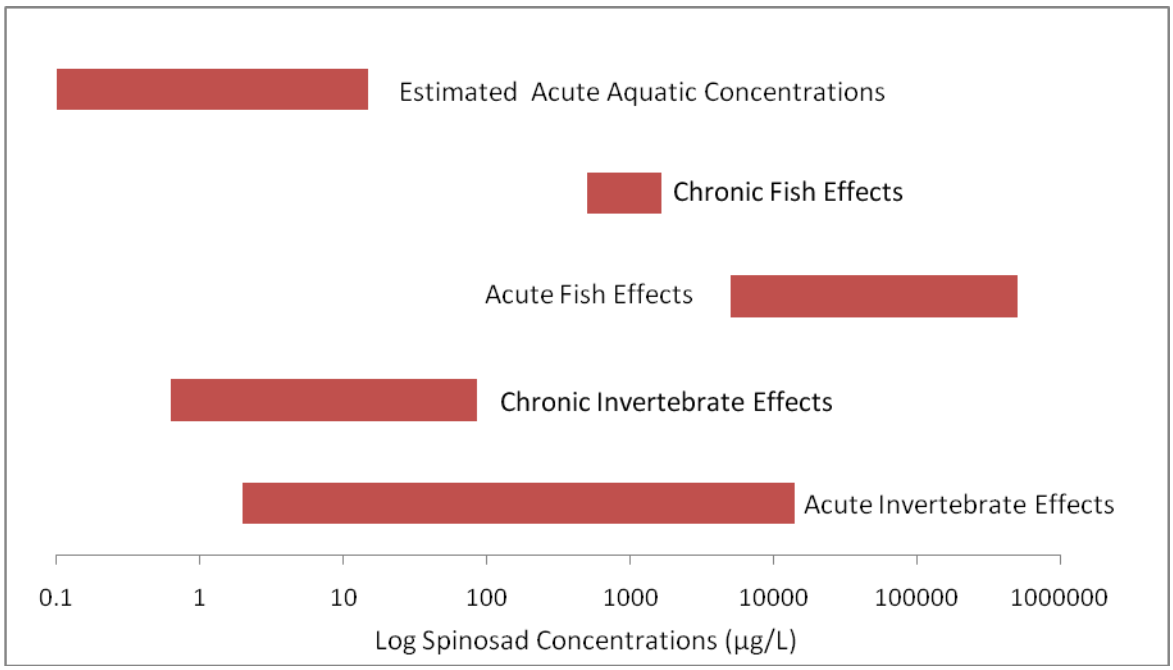

Figure 4. Comparison of edge of field spinosad concentrations and acute and chronic aquatic effects data.

Risk to amphibians would also be expected to be comparable to fish based on the above risk characterization. There is uncertainty in the assumption that fish sensitivity represents amphibian sensitivity; however, based on the very low estimated acute and chronic risks for fish, the difference in sensitivities would have to be several orders of magnitude greater to suggest a presumption of risk under conservative exposure assumptions.

Indirect risk to fish and amphibians can occur through the loss of habitat or prey base. Indirect risk to fish and amphibians through loss of habitat was determined by comparing the lowest available toxicity data for aquatic macrophytes to the estimated aquatic residues. Comparison of the duckweed 14 day EC_{50} value of 10.6 mg/L to the highest edge of field residue (14.8 µg/L) results in an exposure value that is greater than two orders of magnitude below effect thresholds for aquatic macrophytes, suggesting minimal indirect risk of spinosad to fish and amphibian habitat. For some fish and amphibian species indirect risk can occur through impacts to algae that they may depend on as a food source during early development. The lowest algal toxicity value (*Navicula pelliculosa* EC_{50} = 0.107 mg/L) was used to compare to the edge of field maximum reside (14.8 µg/L) and was a factor of ten below the effect threshold suggesting that algal impacts from off-site transport of spinosad would not be expected to occur.

Indirect risk to fish through the loss of prey was assessed by using the range of acute aquatic LC_{50}/EC_{50} toxicity values for invertebrates, and comparing those values to the aquatic residues calculated at edge of field (figure 4). The comparison of acute aquatic invertebrate effects data to the edge of field modeled residues suggests that there could be acute risk to the more sensitive aquatic invertebrates in shallow water habitats. The actual risk is much less as the modeling assumptions assumed 90th percentile estimates of a broadcast application to bare ground. The applications in the EGVM program would not be broadcast over areas of bare ground, but would be directed to host plant material in nursery or noncommercial applications. In addition, the risk

can be reduced by the implementation of a small buffer zone (25 feet) which would reduce exposure in shallow water habitats to below the range of acute invertebrate toxicity values. There was also some overlap in the chronic aquatic invertebrate toxicity data and some of the more shallow water habitats. The actual risk would be much lower because the residue is a maximum instantaneous value and does not assume any degradation or dissipation that would occur due to the short half-life and partitioning of spinosad in the water column. Available field studies testing formulated spinosad impacts on aquatic invertebrates have demonstrated impacts at concentrations ranging from 8 to 33 µg/L in outdoor shallow water mesocosm studies (Duchet et al., 2008). Doses of 17 and 33 µg/L resulted in significant population effects over the duration of the 21-day study; however, *D. pulex* populations recovered to control levels 4 days after treatment due to rapid dissipation of spinosad in the microcosm tanks. The lack of recovery in the higher dosed tanks may be a reflection of enhanced eutrophication that occurred in those tanks as a result of the loss of *D. pulex* in the initial dosing with the resulting poor water quality, which inhibited recovery.

Bacillus thuringiensis var. *kurstaki*

Bacillus thuringiensis var. *kurstaki* (Btk) is a microbial insecticide that belongs to a large group of naturally occurring soil bacteria that have specificity primarily to Lepidoptera (moths and butterflies), but also some Coleoptera (beetles) and Diptera (flies). In the case of the *kurstaki* strain, activity is restricted to lepidopteran larvae. These rod-shaped bacteria produce protein parasporal crystals, which are endotoxins, with activity against certain insects (EPA–1998b). The endotoxin must be ingested by the insect wherein several physiological responses must occur for toxicity to occur. The crystal protein must be solubilized by the highly alkaline midgut (pH 10–11), followed by a proteolytic processing of the protoxin to its active form, which allows binding to the midgut columnar epithelial cells. The toxin is then inserted into the membrane creating pores which causes lysis in the midgut resulting in starvation or septicemia (Whalon and Wingred, 2003; Bravo et al., 2007). The two formulations that are proposed for use are the organic formulations Dipel® DF and Biobit® in either noncommercial applications or nursery treatments using ground equipment.

Exposure Analysis

The environmental fate of Btk is dependent upon whether the emphasis is on the spores or the biologically active endotoxin. Reported half lives for spores in water can range from a few days to greater than a month while soil half lives have been shown to be as long as 200 days (Menon and Menstral, 1985; Hendriksen and Hansen, 2002). The active endotoxin has a much shorter half-life than the spores due to sensitivity to ultraviolet light and breaks down rapidly on foliage with reported foliar half lives ranging from a few hours to approximately four days (Behle et al., 1997; EPA, 1998b; Sundaram et al., 1997; WHO, 1999).

Terrestrial

Exposure to terrestrial receptors was estimated using the maximum use rate proposed for Btk in this program (1.08 lb/ acre), and the assumptions previously described in the drift analysis for spinosad. Point estimates of drift to nontarget terrestrial environments were calculated at the edge of the spray block, and at various distances downwind of the spray block. A point estimate

was used to generate a more conservative drift value, as opposed to integrating the deposition over a given area downwind of the spray block.

Significant drift reductions were estimated based on the previously described application scenario. At 100 feet from the spray block, greater than 99 percent of the drift was reduced with the implementation of the application buffer zone (table 25).

Table 25. AgDrift Point Estimate of Drift and the Reduction of Btk Residues from Applications to Sensitive Terrestrial Habitats.

Chemical	Buffer Zone (ft)	Fraction of Applied	Percent Reduction in Residues
Btk	0	0.9984	-
	25	0.0208	97.9
	50	0.0119	98.8
	75	0.0087	99.1
	100	0.007	99.3

To determine potential residues to contaminated prey for terrestrial vertebrates the T-REX model was used to estimate residues (table 26). The maximum use rate with the smallest application interval was used to provide higher residue levels on potential food items. Due to instability of the endotoxin and short half-life in the field, application intervals can be as short as 3 days.

Table 26. Input Parameters for T-REX Modeling Using Btk.

Parameters	Btk
Application rate (lb ai/ac)	1.08
Half-life (days)	1
Number of Applications	3
Application Interval (days)	3

ai = active ingredient

Based on the selected input parameters the average Btk residues ranged from 28.35 ppm for large insects and seeds to 453.60 ppm on short grass (table 27). The below residues represent upper bound estimates of residues that would be expected from a direct application of Btk.

Table 27. Expected Upper Bound Btk Residues (ppm) on Terrestrial Food Items Using T-REX.

Food Items	Btk Residues (ppm)
Short grass	453.60
Tall grass	207.90
Broadleaf plants/small insects	255.15
Fruits/pods/seeds/large insects	28.35

Using the estimated residues from T-REX doses can be calculated for different mammal classes and body weights to determine the potential range of exposure values that can then be compared to the appropriate effects endpoint (table 28).

Table 28. Dose-based Btk Estimated Environmental Concentrations for Mammals.

Dose-based EEC (mg/kg-bw)	Mammalian Classes and Body Weight					
	Herbivores/Insectivores			Granivores		
	15 g	35 g	1000 g	15 g	35 g	1000 g
Short grass	432.47	298.90	69.30			
Tall grass	198.22	136.99	31.76			
Broadleaf plants/small insects	243.27	168.13	38.98			
Fruits/pods/seeds/large insects	27.03	18.68	4.33	6.01	4.15	0.96

Similar estimates can also be developed for different classes and body weights of birds (table 29). Estimated concentrations ranged from 8.24 ppm for large birds feeding on insects and seeds to 516.60 ppm for small birds feeding exclusively on short grass.

Table 29. Estimated Btk Environmental Concentrations for Several Avian Classes.

Dose-based EEC (mg/kg-bw)	Avian Classes and Body Weights		
	Small 20 g	Mid 100 g	Large 1000 g
Short grass	516.60	294.59	131.89
Tall grass	236.78	135.02	60.45
Broadleaf plants/small insects	290.59	165.71	74.19
Fruits/pods/large insects	32.29	18.41	8.24

Aquatic

Aquatic residues were estimated using AgDrift for a range of aquatic habitats similar to those previously described, with the smaller body of water being 6-inches deep and 10-feet wide. The waterbody was assumed to be static with no evaporation, and all concentrations are instantaneous and do not account for degradation. Residues were highest at edge of field (27.7 µg/L) and then declined to the low ppb range at a buffer of 100 feet (table 30). These modeled residues are considered to be conservative based on the assumptions of the modeling work that was described in the spinosad exposure analysis. These residues do not account for the potential contribution from runoff; however, residues from runoff are expected to be minimal due to the application of Btk directly to foliage, the small area of treatment, and the affinity of Btk to adsorb to soil particles reducing bioavailability. In addition, any buffer related to minimizing disturbance with ground equipment would further minimize Btk runoff by creating a filter strip between the treated vegetation and aquatic habitat.

Table 30. Estimated Btk Residues at Edge of Field and Various Buffer Zones into a Shallow Closed Aquatic Habitat.

Chemical	Buffer Zone (ft)	Aquatic Residues (µg/L)	Percent Reduction in Residues
Btk	0	127.7	-
	25	14.4	88.7
	50	8.8	93.1
	75	6.6	94.8
	100	5.3	95.8

Effects Analysis

Toxicity values for Btk are expressed in several formats which is consistent with other *Bacillus* products. Conventional units, such as mg/kg and mg/L, are used; however, units may be expressed using standard microbiology terms or as activity based on a reference standard preparation. For example, toxicity values may be expressed as colony forming units per liter, or kg, which is typical for use in microbiology. Another more common method of expressing doses in *Bacillus* related studies is by the use of international units per liter or kg. International units (IU) are typically expressed as billion international units (BIU) or as CLU (cabbage looper units). The units are based on the activity of a given *Bacillus* preparation to a standard test species, such as the cabbage looper. For the purpose of this assessment, BIU and CLU are considered equivalent. The application rate proposed in this program is 15.66 CLU/ac based on maximum label recommendations. Data that provides a colony forming unit (CFU) without a conversion to either a weight or international unit measurement was not considered as exposure residues based on label recommendations do not provide concentrations in that format, and would not allow for a comparison between effects and exposure.

Mammalian Toxicity

Mammalian acute and chronic toxicity is low based on the available toxicity data for different formulations of Btk, including the formulations proposed for this program, Dipel® and Biobit®. Acute oral LD_{50} values range from greater than 5,050 mg/kg to greater than 24,600 mg/kg with no adverse effects reported (Valent, 2004; EPA, 1998b). *Bacillus* dermal and inhalation toxicity is also low with dermal LD_{50} values greater than 2,000 mg/L, and inhalation LC_{50} values greater than 2.0 mg/L (Valent, 2004; USDA–FS, 2004). The other ingredients in the formulated products are unknown but compose 46 percent of the formulated material. Although the composition of the other ingredients is proprietary in the proposed formulation, the low toxicity of the formulated product and the registration for organic production suggests that the other ingredients are also low toxicity.

Subchronic and chronic toxicity also appears to be low based on the available mammalian data. Hadley et al., (1987) reported no effects to sheep dosed up to 500 mg/kg/day over during a 5-month exposure, while doses up to 8,400 mg/kg/day did not result in any adverse effects in a 2-year rat study (McClintock et al., 1995). Although all reported toxicity results are greater than the highest test concentration, the lowest LD_{50} value (8,400 mg/kg) and the reported rat NOEL of

8,400 mg/kg/day was used to estimate adjusted toxicity values based on body weight and the percentage of body weight consumed (table 31).

Table 31. Adjusted Btk LD_{50} and NOEL Values for Select Mammals

Mammalian Class	Body Weight (g)	% Body Weight Consumed	Adjusted LD_{50}	Adjusted NOEL
Herbivores/ Insectivores	15	95	18,461.78	18,461.78
	35	66	14,937.55	14,937.55
	1,000	15	6,460.95	6,460.95
Granivores	15	21	1,8461.78	18,461.78
	35	15	1,4937.55	14,937.55
	1,000	3	6,460.95	6,460.95

Avian and Reptile Toxicity

Technical and formulated Btk has low toxicity to birds based on available surrogate data for the bobwhite quail and mallard. Median lethal toxicity values for both species are greater than 2,000 mg/kg with no reported signs of pathogenicity (FS, 2004; EPA, 1998b). No chronic bird toxicity data appears to be available; however, based on the specificity of *Bacillus* and the low acute toxicity to birds, chronic toxicity would not be expected to occur at environmentally relevant doses. The lowest reported LD_{50} value was used to estimate toxicity values based on different body weights and percent of body weight consumed (table 32).

Table 32. Adjusted Btk Acute Toxicity Values for Different Sized Avian Receptors.

Avian Class	Body Weight (g)	% Body Weight Consumed	Adjusted LD_{50}
Small	20	114	1,440.86
Mid	100	65	1,834.29
Large	1,000	29	2,591.00

Toxicity data for reptiles does not appear to be available; however, based on the low toxicity to other vertebrates and specific mode of action of *Bacillus* spores, toxicity to reptiles is expected to be low.

Terrestrial Invertebrate Toxicity

Toxicity to terrestrial invertebrates is low for Btk, with the exception of insects within the lepidopteran order. The selectivity of Btk is based on its previously described mode of action which requires ingestion and solubilization of the endotoxin, which occurs in the midgut of phytophagous lepidopteran larvae that have an alkaline pH midgut. Pollinators, such as honey bees and bumblebees, within the Hymenopteran order are not as sensitive to Btk based on the

available data that demonstrates low toxicity (Sterk et al., 2002). EPA (1998b) reports a 48-hour LD_{50} and NOEL value of greater than 23.2 µg/bee and 7.7 µg /bee, respectively. In longer term studies, the 10-day oral LC_{50} value was reported as 118 µg/bee. Bailey et al. (2005) determined that the contact LD_{50} to formulated Btk to the honey bee (*A. mellifera*) was greater than the highest test concentration (1.0 percent), and that oral exposure to transgenic Btk pollen resulted in no mortality to adult worker bees.

Sensitivity of lepidopteran larvae is dependent on the species and life stage exposed to Btk, and has been evaluated under various laboratory and field conditions. Peacock et al., (1998) evaluated the effects of two Btk formulations to 42 species of nontarget Lepidoptera in laboratory assays. While sensitivity varies based on species and instar tested, significant mortality was observed for species in several families, such as Papilionidae, Nymphalidae, Geometridae, Lasiocampidae, Saturniidae, and Noctuidae.

Herms et al., (1997) evaluated the impacts of formulated Btk on the endangered Karner blue butterfly and found survival rates dropped to 27 percent at a dose rate of 30 to 37 BIU/ha, and 14 percent at a 90 BIU/ha rate. James et al. (1993) evaluated the impacts of Btk on the cinnabar moth (*Tyria jacobaeae*). Sensitivity varied between larval instars with the early instars being less tolerant (LC_{50} = 427-575 BIU/ha) than later instars (LC_{50} = 19-26 BIU/ha). USDA–FS (2004) used the available laboratory terrestrial insect toxicity data to derive dose response relationship curves based on sensitive species that were all lepidopteran, as well as tolerant species which included lepidopteran and other insect taxa. Based on the results of the analysis, a sensitive and tolerant species LD_{50} value of approximately 21 BIU/ha (8.4 BIU/ac) and 590 BIU/ha (240 BIU/ac) was calculated for each group of terrestrial invertebrates.

The range of sensitivities observed in laboratory testing has also been observed in multiple field studies that evaluated the effects of Btk applications to nontarget Lepidoptera. Miller (1990) evaluated the impacts of one Btk application to lepidopteran species richness, species diversity and evenness, larval abundance and a dominance index in a 3-year study where applications were made to 2,000 ha areas for control of gypsy moth. Effects on Lepidopteran species richness and abundance were noted after application; however, effects were varied for individual species. Boulton (2004) evaluated effects after aerial application of Btk to control gypsy moth and found impacts to nontarget lepidopteran in large scale applications in forested areas. Similar impacts have been noted in other studies evaluating the effect of Btk applications on nontarget Lepidoptera (Sample et al., 1996; Wagner et al., 1996; Whaley et al., 1998; Boulton et al., 2002).

Terrestrial Plant Toxicity

No data appears to be available in the literature assessing potential effects of Btk to terrestrial plants. In the re-registration of *Bacillus* products, EPA waived this study requirement after a review of the available information suggested plants would not be adversely affected by *Bacillus* applications (EPA, 1998b). As previously described, the mode of action and requirement for solubilization at an alkaline pH after ingestion by an insect would support the assumption that no phytotoxicity would be expected.

Aquatic Toxicity

Several aquatic toxicity values are available for Btk, as well as other *Bacillus* insecticides. Several of these values are reported as CFU/L and are not readily convertible to a concentration that can be compared to exposure levels unless conversions are presented. Field studies also report concentrations as billion international units (BIU)/L which can be converted to mg/L. Emphasis in this risk assessment will be placed on those toxicity values that are represented in mg/L or for studies where the conversion from cfu/L or BIU/L to mg/L can be made based on adequate descriptions in the material and methods section of relevant papers.

EPA (1998b) reported that Btk is considered practically nontoxic to the bluegill, rainbow trout, and sheepshead minnow with acute toxicity exceeding the highest value tested. Concentrations are represented in cfu/L, and are reported as being greater than 10^{10} cfu/L. Mayer and Ellersiek (1986) reported an LC_{50} value of greater than 10 mg/L for the rainbow trout and 95 mg/L for the bluegill. Reports for Dipel® and Biobit® formulations suggest low toxicity with toxicity greater than 102 mg/L for those studies where concentrations can be converted to mg/L (USDA–FS, 2004; EPA, 2010)

USDA–FS (2004) reports a 96-hour NOEC value of 1,000 mg/L (2.5×10^{10} cfu/L) for the mosquito fish, and NOEC of 1.4 mg/L (2.87×10^{7} cfu/L) for the rainbow trout in a 30-day exposure based on conversions of cfu/L to mg/L from information available in the study reports that were submitted to support registration. The effect was not attributed to direct toxicity from but from the excessive competition for food that resulted from poor visibility due to presence of excessive suspended solids (WHO, 1999). WHO (1999) reported no effects to bluegill or the sheepshead minnow in 30- or 32-day exposures to 2.9×10^{9} cfu/L and 2.6×10^{10} cfu/L, respectively, of formulated Btk. Some toxicity data appears to be available regarding the impacts of Btk to amphibians. WHO reports that in a review of available data there were no adverse effects reported for Btk to frogs (*Hyla regilla*, *Rana temporaria*), toads *(Bufo* species), or newts (*Taricha torosa*, *Triturus vulgaris*); however, test concentrations were not reported (WHO, 1999). Based on the limited data for amphibians and the available toxicity data for fish, Btk toxicity is expected to be low for amphibians.

Toxicity to aquatic invertebrates from Btk is variable but would be considered low for most test organisms in acute and chronic exposures. The 48-hour EC_{50} is greater than 102 mg/L for the freshwater cladoceran, *D. magna* (EPA, 2010). In 21-day toxicity tests the LC_{50} for *D. magna* is reported as ranging from 5 to 50 mg/L (EPA, 1998b). Toxicity to the grass shrimp, *Palaemonetes vulgaris*, is low with reported lethal and sublethal values higher than the highest test concentration based on aqueous (4.9 µl/L) and dietary exposures (2.9×10^{9} cfu/g) (EPA, 1998b). Chronic studies using *D. magna* reveal effects on reproduction at 5.9 mg/L with a reported NOEC of 0.45 mg/L or 6.24×10^{8} cfu/L (USDA–FS, 2004).

Chronic sediment toxicity appears to be low based on results from a static exposure study using the marine copepod, *Amphiascus minutus*, with a reported NOEL of 500 mg/kg (EPA, 1998b).

Eidt (1985) tested the effects of formulated Btk to several aquatic insects, including larvae of Simuliidae, Chironomidae, Trichoptera, Megaloptera, and nymphs of Ephemeroptera and Plecoptera. Insects were exposed to three test concentrations (4.3, 43, and 430 IU/mL), with the

lowest concentration representing twice the worst-case exposure concentration of 15 µg/L from monitoring studies after aerial forestry applications. Of the eight insect species tested, only blackfly (Simuliidae) larvae were impacted with effects occurring at the 430 IU/mL concentration but not at the lower concentrations which were approximately 2 and 20 times above the estimated worst-case field residues. The 430 IU/mL concentration corresponds to a concentration of approximately 50 mg/L; therefore, the NOEC for blackflies was 5 mg/L. Kreutzweiser et al. (1992) found a similar lack of lethal and sublethal effects to insects in the order Ephemeroptera, Plecoptera, and Trichoptera. Toxicity test results for six species of Ephemeroptera, three species of Plecoptera, and four species of Trichoptera demonstrated LC_{50} values greater than 600 IU/mL for all test organisms. Tests in outdoor stream channels using the same species tested at 600 IU/mL showed no Dipel®-related effects on invertebrate drift or mortality. Kreutzweiser et al. (1994) evaluated laboratory and field of effects of Btk applications on the stonefly, *Leuctra tenuis*, at 10 times the expected environmental concentrations (200 IU/mL), and also assessed impacts to benthic stream communities. Some effects related to reduced abundance were noted for *L. tenuis* in the field but not in laboratory exposures. Kreutzweiser and Capell (1996) also found no effects on mortality or palatability of dosed leaf discs to the detritivorous caddisfly, *Hydatophylax argus*, after exposures to Dipel® at 20 and 20,000 IU/mL, which was 20 and 1,000 times the expected estimated environmental concentration resulting from direct application to water. Richardson and Perrin (1994) evaluated the impacts of formulated Btk on stream benthic communities in a stream mesocosm by dosing at a rate of 50 and 500 BIU/ha, which resulted in aqueous mean concentrations of 2.1×10^4 and 1.8×10^8 cfu/mL, respectively, during treatment. These rates represent greater than 100 times the expected residues from a direct application to water. Elevated drift rates for *Baetis* were noted immediately after application, but returned to levels comparable to the controls within 24 hours. No differences were noted in the density or composition of benthos nor, were any differences noted in emergence rates between control and treated mesocosms.

Toxicity to aquatic plants is limited to one study that was conducted using the crystal proteins from a Dipel® formulation against pure and mixed algal cultures containing *Euglena*, *Chlamydomonas*, *Oedogonium*, and a cyanobacterium (*Oscillatoria* sp.). Test concentrations of the protein are not stated in the publication; however, the authors state there was no inhibitory activity to any of the pure or mixed algal cultures (Koskella and Stotzky, 2002). Toxicity is not expected based on the specificity of Btk to certain insects, and the requirements needed for the endotoxin activation which is not present in aquatic plants.

Risk Characterization

Terrestrial (Mammals, Birds and Reptiles)

Direct risk to mammals was determined by comparing the lowest available acute and chronic toxicity data points to the estimated residues that would be expected from applications of Btk. Direct risk to select mammal groups is considered minimal based on estimated RQ values (table 33).

Table 33. Dose-based Risk Quotient Values for Select Mammal Groups.

Dose-based RQs (Dose-based EEC/LD_{50} or NOEL)	15 g mammal		35 g mammal		1,000 g mammal	
	Acute	Chronic	Acute	Chronic	Acute	Chronic
Short grass	0.02	0.02	0.02	0.02	0.01	0.01
Tall grass	0.01	0.01	0.01	0.01	<0.01	<0.01
Broadleaf plants/small insects	0.01	0.01	0.01	0.01	0.01	0.01
Fruits/pods/large insects	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Seeds (granivore)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

The estimates of risk are conservative as they are based on toxicity endpoints that are reported as greater than the highest test concentration. In addition, the estimates of exposure are based on the assumption that mammals will only consume contaminated forage or prey which would not be the case in this program because applications are restricted to small areas, and any foraging mammals that could consume contaminated food items would forage outside the treatment area, as well.

Estimated direct risk to representative avian groups is also considered low based on the estimated RQ values (table 34). Similar to mammals, the estimates of risk to birds would also be low as toxicity to birds is above the limit for testing, and the exposure assessment assumes that birds from each group will forage only on contaminated forage and prey. This would not be the case in this program due to the small areas of treatment. The low risk to avian species is also assumed for reptiles based on the lack of toxicity to all vertebrates, the specificity of the endotoxin to certain insect groups, and lack of significant exposure.

Table 34. Dose-based Risk Quotient Values for Select Avian Species.

Dose-based RQs (Dose-based EEC/LD_{50})	Avian Acute RQs		
	20 g	100 g	1000 g
Short grass	0.36	0.16	0.05
Tall grass	0.16	0.07	0.02
Broadleaf plants/small insects	0.20	0.09	0.03
Fruits/pods/large insects	0.02	0.01	<0.01

Indirect risk to mammals, birds, and reptiles are related to loss of habitat or prey resulting from Btk applications. Loss of habitat through impacts on terrestrial plants is not expected based on the specific mode of action for *Bacillus*, and lack of phytotoxicity reported by its use in a variety of aquatic and terrestrial applications. Indirect risk through loss of terrestrial invertebrate prey is also not expected based on the method of application proposed for this program, the available invertebrate toxicity data, and studies that have been conducted to evaluate indirect effects to terrestrial vertebrates after *Bacillus* applications. While some indirect impacts have been noted, particularly in birds from the loss of prey items, these types of impacts have only occurred in large scale broadcast applications in forestry situations, and are not representative of the

proposed use pattern in the control of EGVM (Burgess et al., 1995). Summaries of some of these studies are presented below for mammals and birds.

Belloco et al. (1994) evaluated the effects of Btk applications to the diet of the masked shrew, *Sorex cinereus* in Jack Pine (*Pinus banksiana*) plantations after aerial applications of Dipel® to a 44-ha treatment site. No difference in abundance of shrews was observed between the treated and control plot; however, there were more juveniles in the treated area compared to the controls after treatment. As would be expected, there were less Lepidoptera available in the treated area than the control; however, juveniles and adults shifted to alternative invertebrate prey, and the authors concluded that Btk applications would have minimal impacts to generalist insectivores. This type of shift would not be expected in this program because the application area is much smaller and small mammals would forage inside and outside the areas of treatment. A lack of effects to small mammal populations was also observed in another field study where aerial applications of Thuricide, another Btk formulation, at a rate of 20 BIU/ha did not result in effects on the abundance of small rodents and shrews (Innes and Bendell, 1989).

Norton et al. (2001) assessed the impacts of aerial Btk. applications on spruce grouse, (*Dendragapus canadensis*) chicks on 100-ha treatment plots at application rates greater than three times the rates proposed in this program. Reduced growth rates were noted in chicks on treatment plots compared to those on the control plots in Jack pine (*P. banksiana*) forests. Sopuck et al. (2002) evaluated the indirect impacts of aerial Btk applications to songbirds during applications in Canada to a greater than 12,000-ha area over a 2-year period. The authors evaluated abundance, richness, and broods for over 40 species of songbirds to determine potential indirect effects from reductions in prey. The species evaluated represented a distribution of feeding guilds ranging from omnivores to leaf-gleaning insectivores. With the exception of one species, the spotted towhee (*Papilo maculates*) in 1 year only, there were no effects from Btk applications on the number of broods. Holmes (1998) evaluated the indirect effects of Btk applications to reproduction and nesting behavior for the Tennessee warbler, *Vermivora peregrine*, which are important predators of the spruce budworm. No significant differences were found for nestling survival, growth, or diet between control and treated plots. Similar results have been reported for the black-throated blue warblers, *Dendroica caerulescens*, where no effects were observed for endpoints, such as clutch size, hatching or fledging success, as well as annual breeding productivity. Second broods were less common in treated blocks compared to the control blocks (Rodenhouse and Holmes, 1992). Marshall et al. (2002) evaluated similar parameters in a 2-year study using treated and untreated 30-ha blocks to evaluate effects to the red eyed vireo (*Vireo olivaceus*). No effects were noted in clutch size, nest and hatching success, or nestling mortality during the study. The authors did state that birds in treated areas waited 3 to 5 days longer in treated sites to initiate nest construction, but it was unclear whether this was due to natural or experimental causes.

Nagy and Smith (1997) evaluated the impact of field Btk applications on several reproductive parameters for the hooded warbler, *Wilsonia citrina*, for 2 years after treating plots ranging in size from 15 to 80 ha. The authors concluded that while there was a reduction in lepidopteran larvae, there was little impact on reproduction of hooded warblers.

Direct and indirect effects to terrestrial vertebrate species are unlikely with the proposed program use of Btk. Laboratory and field data support the lack of direct effects and, in rare cases, where

indirect effects were noted they occurred in studies where application rates were typically greater than those proposed in the EGVM program, and over significantly larger treatment areas than proposed in this program.

Terrestrial (Plants and Invertebrates)

Direct risks to terrestrial plants are not expected based on the mode of action of Btk which is specific to certain insects primarily in the order Lepidoptera. The potential for indirect risk to terrestrial plants is also considered minimal based on the limited area of proposed treatment, a lack of toxicity to most pollinators, and the mode of action that is specific to lepidopteran larval stages.

Risks to terrestrial invertebrates are primarily confined to nontarget lepidoptera larvae that may ingest the endotoxin. The risk to endangered Lepidoptera is minimized based on label recommendations for Dipel® DF that state "No manual application can be made within 300 feet of any threatened or endangered Lepidoptera." Based on modeling results from the proposed application, this would result in a 99.72 percent reduction of residues using conservative off-site drift assumptions. To assess whether this reduction would result in residues that are below potential effects, the toxicity estimate for sensitive insect species (8.4 BIU/ac) was compared to the reduction in residues that would be expected from using application buffers from sensitive lepidopteran habitat. The application rate or exposure level proposed for this program is 15.66 billion CLU, or BIU, of Btk per acre based on label recommendations. When the 99.72 percent reduction in drift is applied to the rate expected within the treated area, the residue value is reduced to 0.043 CLU/ac. The exposure value is greater than 195 times below the sensitive effect value suggesting substantial reduction in risk with the implementation of the required 300-foot buffer.

Aquatic

Direct acute and chronic risk to fish from proposed Btk applications based on conservative estimates of exposure in a range of aquatic habitats is expected to be minimal (figure 5). Residues in the small enclosed habitat were approximately two orders of magnitude below the lowest reported acute fish toxicity value. Risk would actually be much less as the value was reported as greater than the highest test concentration tested (> 10 mg/L). The chronic risk to fish is also considered conservative because the toxicity results are based on 30-day exposures, and effects were not attributed to direct toxicity from Btk. A lack of direct risk is also expected for amphibians. A summary of reported acute toxicity data for amphibians states no lethal or sublethal impacts at similar levels to the available fish toxicity data. The uncertainty in this assessment is the lack of the actual reported dosing levels; however, based on the available fish data for Btk, the reported lack of effects in other studies, and the specificity of Btk in its mode of action to certain insects, the direct risk to amphibians is expected to be minimal.

Estimated residues for Btk were well below the range of acute and chronic effects data for aquatic invertebrates. Although indirect risks to fish have not been evaluated in field studies, the lack of toxicity to aquatic invertebrates from various laboratory and field studies suggest that indirect risk through the loss of prey are not expected. Indirect risk to fish through loss of habitat and food from toxicity to aquatic plants is also not expected based on the specific mode of action

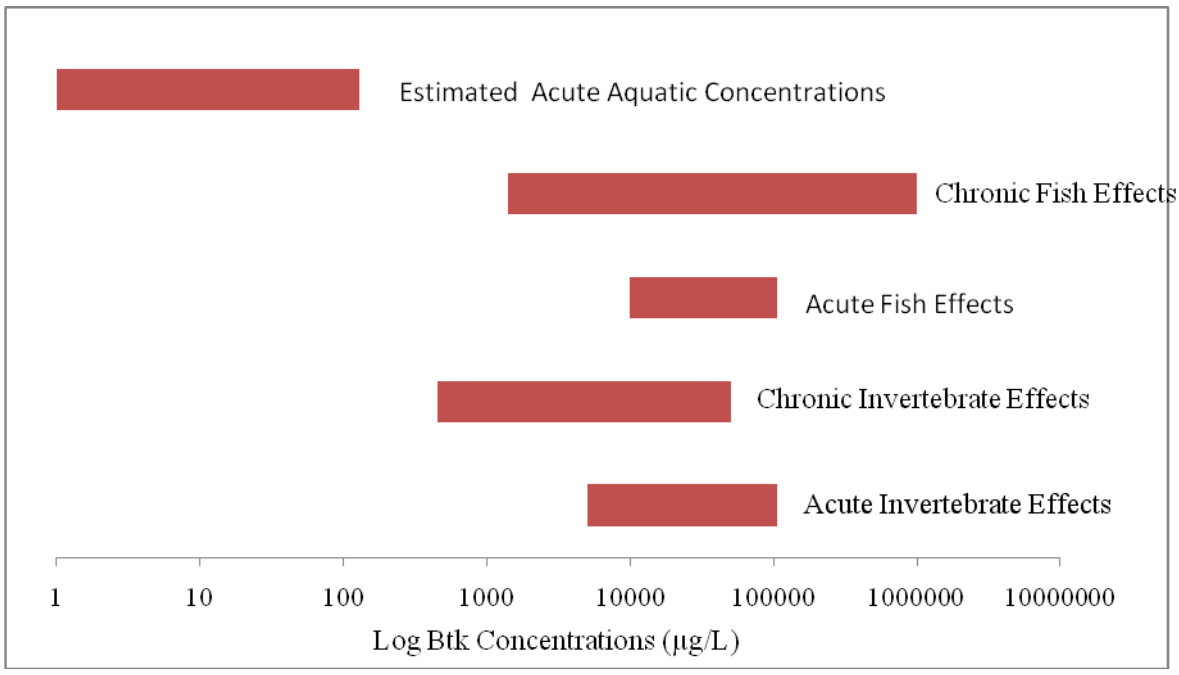

Figure 5. Comparison of edge of field Btk concentrations and acute and chronic aquatic effects data.

for the endotoxin. Impacts to amphibians from the loss of prey have been evaluated in field conditions for several salamander species. Raimondo et al. (2003) evaluated the indirect effects of formulated Btk applications at a rate of 16 BIU/ha in the Monongahela National Forest in West Virginia over a 2-year period. No effects on salamander abundance or the proportion of major prey items for five salamanders (Plethodontidae (*Desmognathus fuscus*, *D. monticola*, *D. ochrphaeus*, *Plethodon cinereus*, and *P. glutinosus*) was noted during the study.

Methoxyfenozide

Methoxyfenozide is an insect growth regulator (IGR) in the diacylhydrazine class of insecticides that functions as an ecdysone agonist causing disruption of the molting process by serving as a mimic for 20-hyrodoxyecdysone (figure 5). The primary mode of action occurs through ingestion with activity against larval lepidoptera.

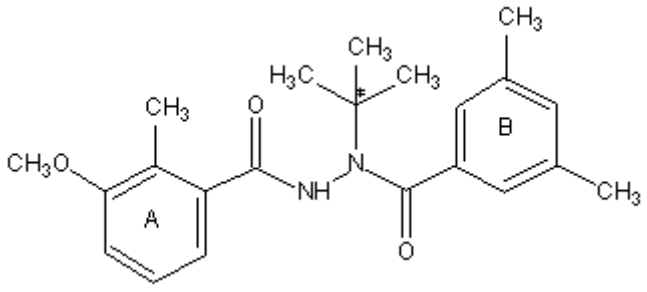

Figure 5. Chemical structure for methoxyfenozide.

The formulation proposed for use in this program is Intrepid®, which is applied by ground using a backpack sprayer or other ground equipment.

Exposure Analysis

Methoxyfenozide has low solubility in water with a reported value of 3.3 mg/L. Methoxyfenozide is considered stable in the presence of water, light, and microbial activity under laboratory and field conditions. Half-life values range from 173 days in a soil photolysis study to 1,100 days in an aerobic soil metabolism study (PMRA, 2004) (table 35).

Table 35. Environmental Fate Parameters for Methoxyfenozide.

Environmental Fate Parameter	Reported Half-life (days)
Hydrolysis	pH 5: 587, pH 7: 1572, pH 9: 695
Aqueous Photolysis	Stable
Soil Photolysis	173
Aerobic Soil Metabolism	336–1100
Anaerobic Aquatic Metabolism	654
Aerobic Aquatic Metabolism	387–963
Terrestrial Field Dissipation	268–433

Depending on the soil types, methoxyfenozide may be subject to movement as runoff. Soil adsorption coefficients range from approximately 219 to 365 in loam, loamy sand, sandy loam and silt loam with a large range in mobility reported in loamy sand soils (200.2- 922). Volatility is not expected to be a major pathway of exposure based on the low reported vapor pressure < 1.33×10^{-5} Pa.

Terrestrial

Exposure estimates for terrestrial vertebrate food items were estimated using the T-REX model. Input parameters such as application methods and environmental fate parameters were selected to maximize potential residues (table 36). Application rates and intervals are based on information provided on the Intrepid® 2F Section 3 label for the grape berry moth or supplemental label for use in California for EGVM.

Table 36. Input Parameters for T-REX Modeling

Parameters	Methoxyfenozide
Application Rate (lb/ai/ac)	0.25
Half-life (days)	433
Number of Applications	3
Application Interval (days)	10

Upper bound 90th percentile residues on a variety of terrestrial food items ranged from 11.07 ppm on items, such as seeds, pods, and large insects to 177.16 ppm, on short grass (table 37). All residues are based on direct applications to each of the food items.

Table 37. Expected Upper-bound Methoxyfenozide Residues (ppm) on Terrestrial Food Items using T-REX.

Food Items	Methoxyfenozide Residues (ppm)
Short grass	177.16
Tall grass	81.20
Broadleaf plants/small insects	99.65
Fruits/pods/seeds/large insects	11.07

The values listed above can be used to calculate exposure concentrations based on mg/kg body weight, and adjusted for different receptor classes using known ingestion rates. Mammal body weights, ingestion rates, and percent of body weight consumed values are listed below for a representative herbivore, insectivore, and granivore mammal (table 38).

Table 38. Adjusted Mammal Parameters for Different Class and Body Size.

Mammalian Class	Body Weight (g)	Ingestion (dry) (g bwt/day)	Ingestion (wet) (g/day)	% Body Weight Consumed	Food Intake (kg-diet/day)
Herbivores/ Insectivores	15	3	14	95	1.43E-02
	35	5	23	66	2.31E-02
	1,000	31	153	15	1.53E-01
Granivores	15	3	3	21	3.18E-03
	35	5	5	15	5.13E-03
	1,000	31	34	3	3.40E-02

Using these values, estimated environmental concentrations (EECs) can be calculated based on the dose that each type of mammal would receive from different food items (table 39). This value can then be compared to the adjusted LD_{50} and NOEL values to determine if exposure levels exceed effect thresholds.

Table 39. Dose-based Methoxyfenozide Estimated Environmental Concentrations for Mammals.

Dose-based EEC (mg/kg-bw)	Mammalian Classes and Body Weight					
	Herbivores/Insectivores			Granivores		
	15 g	35 g	1000 g	15 g	35 g	1000 g
Short grass	168.91	116.74	27.07			
Tall grass	77.41	53.50	12.41			
Broadleaf plants/small insects	95.01	65.66	15.22			
Fruits/pods/seeds/large insects	10.56	7.30	1.69	2.35	1.62	0.38

Doses for birds can also be calculated based on body weight and consumption rates for a range of bird sizes (table 40). For this assessment, birds ranging in size from 20 to 1000 g were used to calculate the amount of food that could be consumed in a given day.

Table 40. Adjusted Avian Parameters for Different Class and Body Size.

Avian Class	Body Weight (g)	Ingestion (Fdry) (g bw/day)	Ingestion (Fwet) (g/day)	% Body Weight Consumed	(kg-diet/day)
Small	20	5	23	114	2.28E-02
Mid	100	13	65	65	6.49E-02
Large	1000	58	291	29	2.91E-01

Dose-based EECs can also be made for different avian groups so that doses based on body weight and feeding preference can be compared to the adjusted toxicity effects endpoint (table 41). These comparisons are discussed in the risk characterization section of this risk assessment.

Table 41. Estimated Methoxyfenozide Environmental Concentrations for Several Avian Classes.

Dose-based EEC (mg/kg-bw)	Avian Classes and Body Weights		
	small 20 g	mid 100 g	large 1000 g
Short grass	201.76	115.05	51.51
Tall grass	92.48	52.73	23.61
Broadleaf plants/small insects	113.49	64.72	28.98

Aquatic

Edge of field residues for methoxyfenozide ranged from the low ppt range in the larger waterbody that was modeled to the low ppb range in the shallow aquatic habitat that was modeled. In the shallow water habitat, the effect of application buffers was assessed to

determine the reduction of drift residues at different buffer distances (table 42). Within the first 25 feet, approximately 89 percent of the available methoxyfenoxide drift is removed from the aquatic habitat. There is a reduction in off-site drift into the shallow aquatic habitat as the buffer is increased; however, the amount of reduction in drift after the first 25 feet drops significantly. Limitations in the model do not allow a 100-percent reduction in residues. Current application restrictions require a 25-foot setback from most aquatic habitats.

Table 42. Estimated Methoxyfenozide Residues at Edge of Field and At Various Buffer Zones in a Shallow Closed Aquatic Habitat.

Chemical	Buffer Zone (ft)	Aquatic Residues (µg/L)	Percent Reduction in Residues
Methoxyfenozide	0	29.3	-
	25	3.1	89.4
	50	1.8	93.8
	75	1.3	95.6
	100	1.0	96.6

Effects Analysis

Mammalian Toxicity

Available mammalian toxicity data for methoxyfenozide using the rat, mouse, and rabbit suggest low toxicity from oral, dermal, and inhalation exposure pathways. Acute oral, dermal, and inhalation toxicity from exposure to the technical and the formulation suggest low toxicity (table 43) (WHO, 2003; Dow AgroSciences, 2008).

Table 43. Comparative Acute Mammalian Toxicity of Methoxyfenozide and Intrepid.

Test	Technical	Intrepid®
Oral LD_{50}	>5000 mg/kg	>5000 mg/kg
Dermal LD_{50}	>5000 mg/kg	>2000 mg/kg
Inhalation LC_{50}	>4.3 mg/L	>0.9 mg/L

Subchronic and chronic effects data for methoxyfenozide reveal low toxicity based on a variety of tests and different surrogate species (table 44). NOEL values in 90-day exposures were the highest test concentration tested. Methoxyfenozide is not considered a mutagen, carcinogen, teratogen, or reproducting toxicant based on available mammalian toxicity studies (PMRA, 2004).

Table 44. Subchronic and Chronic Mammalian Toxicity Values for Methoxyfenozide.

Toxicity Test/ Test Species	NOEL (mg/kg/day)
90-day/Rat	1369
90-day/Mouse	1149
90-day/Dog	198
Chronic/Carcinogenicity 2-yr/Rat	10.2
Carcinogenicity 18-month/Mouse	1020
Developmental Toxicity/Rat	1000
Developmental Toxicity/Rabbit	1000
Reproductive Toxicity/Rat	153

Based on the available acute and chronic toxicity values for mammals estimates of toxicity can be made for other types of mammals that have varying sizes and feeding rates. For the adjusted acute dose estimates, the lowest LD_{50} (5,000 mg/kg) was used while for the chronic data the lowest reported reproductive NOEL (153 mg/kg/day) was used to estimated chronic doses for a variety of mammals (table 45).

Table 45. Adjusted LD_{50} and NOEL Values for Select Mammals.

Mammalian Class	Body Weight	% Body Weight Consumed	Adjusted LD_{50}	Adjusted NOEL
Herbivores/ Insectivores	15	95	10,989.15	336.27
	35	66	8,891.40	272.08
	1,000	15	3,845.80	117.68
Granivores	15	21	10,989.15	336.27
	35	15	8,891.40	272.08
	1,000	3	3,845.80	117.68

Avian and Reptile Toxicity

Avian toxicity to methoxyfenozide is also low based on standardized acute oral and dietary studies with the mallard and bobwhite quail (EPA, 2010). Acute oral and dietary studies report toxicity values above the highest test dose suggesting that methoxyfenozide is practically non-toxic to birds based on available data. Sublethal effects were not observed at the highest test concentration, with the exception of the dietary mallard study which reported a NOEL value of 562 ppm (table 46).

Table 46. Acute and Chronic Avian Toxicity of Methoxyfenozide.

Test Species/ Duration	LD_{50}/LC_{50} (mg/kg)	NOEL/LOEL (mg/kg)
Bobwhite quail LD_{50}	>2250	2250/NR
Bobwhite quail LC_{50}	>5620	5620/NR
Bobwhite quail chronic reproduction	NR	520/780
Mallard LC_{50}	>5620	562/NR
Mallard chronic reproduction	NR	780/1000

NR = Not reported

Using the lowest avian acute NOEL adjusted values were estimated for birds with different body weights and percent of body weight consumed to establish a range of sensitivities (table 47).

Toxicity data for reptiles does not appear to be available as these types of studies are not a condition of registration. Assumptions regarding the comparative toxicity between reptiles and birds have some uncertainty; however, this will be discussed in the risk characterization section of this assessment.

Table 47. Adjusted Acute Toxicity Values for Different Sized Avian Receptors.

Avian Class	Body Weight (g)	% Body Weight Consumed	Adjusted NOEL
Small	20	114	1620.97
Mid	100	65	2063.57
Large	1000	29	2914.87

Terrestrial Invertebrate Toxicity

Nontarget toxicity testing using the honey bee shows low toxicity after exposure to methoxyfenozide. The acute and oral contact LD_{50} for the honey bee is greater than 100 µg/bee, with a corresponding NOEL of 100 µg/bee, suggesting methoxyfenozide is practically nontoxic in acute contact exposures (EPA, 2010; PMRA, 2004). Impacts to other nontarget invertebrates, other than Lepidoptera, have shown a similar lack of impacts at relevant doses. Mommaerts et al. (2006) exposed the bumblebee, *Bombus terrestris*, to a formulation of methoxyfenozide, Runner® by contact, as well as orally by dosing sugar water and pollen, found no impacts on adult survival, nest reproduction, or larval growth. A majority of the other terrestrial nontarget invertebrate work to date with methoxyfenozide has targeted beneficial insects. Studies examining the impacts of methoxyfenozide on hymenopteran parasitoids, predacious bugs, have demonstrated varying impacts to beneficial insects (Hewa-Kapuge, et al., 2003; Kim et al., 2006).

Terrestrial Plant Toxicity

Pesticide registration of insecticides typically does not require toxicity testing for terrestrial plants. A review of the literature regarding the efficacy of methoxyfenozide in various row crop and orchard applications does not report any adverse impacts to plants at application rates above those proposed in this program. Based on the mode of action of methoxyfenozide and its specificity to insects, nontarget terrestrial phytotoxic effects are not anticipated.

Aquatic Toxicity

Available acute and chronic toxicity fish data suggests that methoxyfenozide toxicity is low. Acute lethal and sublethal values were greater than reported solubility values for methoxyfenozide (EPA, 2010) (table 48).

Table 48. Methoxyfenozide Toxicity to Aquatic Vertebrates

Test Species/ Duration	LC_{50}/EC_{50} (mg/L)	NOEC/LOEC (mg/L)
Acute Tests		
96-hour LC_{50} Bluegill Sunfish	>4.3	4.3
96-hour LC_{50} Rainbow Trout	>4.2	4.2
96-hour LC_{50} Sheepshead Minnow	>2.8	2.8
Subchronic/Chronic Tests		
Fathead minnow 31-d	NR	2.4/>2.4
Fathead minnow 262-d	NR	0.53/1.0
Sheepshead 35-d ELS*	NR	1.5/2.6

*ELS = Early life stage study
NR = Not reported

Aquatic toxicity to invertebrates was variable, depending on the test species and duration of the exposure. Aquatic insects, such as the midge, *Chironomus*, appear to be the more sensitive aquatic invertebrates based on acute and longer term exposures (EPA, 2010) (table 49).

Table 49. Methoxyfenozide Toxicity to Aquatic Invertebrates.

Test Duration/Species	LC_{50}/EC_{50} (mg/L)	NOEC/LOEC (mg/L)
Acute Tests		
48-hour EC_{50} *Daphnia magna*	3.7	1.7/NR
96-hour LC_{50} Mysid Shrimp	1.3	0.68/NR
96-hour LC_{50} Eastern Oyster	1.2	0.40/NR
5-day LC_{50} *Chironomus tentans*	0.62	NR/NR
10-day LC_{50} *Aedes aegypti*	12.85	NR/NR
10-day LC_{50} *Culex quinquefasciatus*	3.12	NR/NR
10-day LC_{50} *Anopheles gambiae*	2.75	NR/NR
Chronic Tests		
21-day LC_{50} *Daphnia magna* (life cycle)	NR	0.20/0.39
28-day LC_{50} *Chironomus sp.*	NR	0.0063/0.012
21-day *Culex quinquefasciatus*	0.21	NR/NR
37-day Mysid Shrimp (life cycle)	NR	0.025/0.051

NR = Not reported

No data appears to be available regarding the acute or chronic toxicity of methoxyfenozide to amphibians and aquatic plants. Assuming that the fish toxicity values are representative of the sensitivity to amphibians, the toxicity would be expected to occur only at levels above solubility. Toxicity to aquatic plants would also be expected to be low due to the mode of action of methoxyfenozide, which is specific to invertebrates, and the lack of phytotoxicity to terrestrial plants (PMRA, 2004).

Risk Characterization

Terrestrial (Mammals, Birds and Reptiles)

The acute and chronic risk to wild mammals is low based on the upper bound residues that could occur on a variety of food resources for mammals that receive the maximum labeled application of methoxyfenoxide (table 50). Risk to mammals is actually much lower than estimated in the table below as the effects endpoints that were selected for the acute and chronic values were studies wherein the effect endpoint was greater than the highest test concentration.

Table 50. Dose-based Risk Quotient Values for Multiple Mammal Classes.

Dose-based RQs (Dose-based EEC/LD50 or NOEL)	15 g Mammal		35 g Mammal		1000 g Mammal	
	Acute	Chronic	Acute	Chronic	Acute	Chronic
Short grass	0.02	0.50	0.01	0.43	0.01	0.23
Tall grass	0.01	0.23	0.01	0.20	<0.01	0.11
Broadleaf plants/small insects	0.01	0.28	0.01	0.24	<0.01	0.13
Fruits/pods/large insects	<0.01	0.03	<0.01	0.03	<0.01	0.01
Seeds (granivore)	<0.01	0.01	<0.01	0.01	<0.01	<0.01

Acute direct risk to avian species is also considered low with exposure levels well below the lowest NOEL value that has been reported in acute toxicity studies with birds (table 51).

Table 51. Dose-based Risk Quotient Values for Multiple Avian Classes.

Dose-based RQs (Dose-based EEC/NOEL)	Avian Acute RQs		
	20 g	100 g	1000 g
Short grass	0.12	0.06	0.02
Tall grass	0.06	0.03	0.01
Broadleaf plants/small insects	0.07	0.03	0.01
Fruits/pods/large insects	0.01	<0.01	<0.01

No adverse impacts were noted at the reported NOEL value; therefore, the above risk estimates overestimate actual risk. Chronic risk to birds from methoxyfenozide exposure is also considered low. A comparison of the lowest reported NOEC value from the bobwhite quail reproductive study (520 ppm) and to the range of upper bound estimates of exposure concentrations on various food items (11.04–176.64 ppm) demonstrated RQ values ranging from 0.02 to 0.34.

Direct risk to reptiles is also considered low based on the estimates derived for birds. Reptile sensitivity to methoxyfenozide would have to be at least two orders of magnitude greater compared to birds for any potential risk to occur.

Indirect risks to terrestrial vertebrates that depend on terrestrial invertebrates for food are also expected to be minor because the areas of application proposed in this program are small. Plants within the 500-meter treatment radius will be treated using ground equipment which will reduce drift when compared to an aerial broadcast application. Applications in nursery settings could result in potentially greater nontarget impacts to terrestrial invertebrates as those areas of treatment may be larger. However, in both cases, broad spectrum impacts to all terrestrial invertebrates are not expected due to the mode of action of methoxyfenozide and selective toxicity to certain life stages and species of insects. In addition, the typical small area of treatment compared to the foraging range of most terrestrial vertebrates will enable those

vertebrates to forage in adjacent areas, and will also allow immigration of any impacted invertebrates into treated areas.

For those terrestrial vertebrates that depend on aquatic invertebrates or fish, no indirect impacts would be expected based on the characterization of risk to aquatic biota which is discussed in the next section. Exposure from the ingestion of contaminated aquatic prey items is also not expected based on the lack of bioconcentration reported in fish. In a 28-day exposure using bluegill sunfish, the highest bioconcentration factor was 8.9 to 23 in viscera, and 8.9 in whole fish (PMRA, 2004). The depuration half-life was reported as less than a half a day, indicating that methoxyfenozide is rapidly eliminated from fish.

Terrestrial (Plants and Invertebrates)

Direct risks to terrestrial plants are not expected because methoxyfenozide is not considered phytotoxic. Indirect risk to terrestrial plants that are insect-pollinated is also expected to be low in most cases due to the lack of broad spectrum activity against different life stages and types of different insect pollinators, and the relatively small area of treatment for most treatments. Lethal and sublethal toxicity of methoxyfenozide to bees is reported as low; however, some impacts have been noted for some beneficial insects, as well as the target lepidoptera, that could serve as pollinators for certain plants. In the case of rare and listed plants, an application buffer may be required if applications are expected to occur during pollination.

Aquatic

Acute and chronic risk is low for fish based on edge of field methoxyfenozide residues estimated for a variety of aquatic habitats, and comparing those values to the available acute and chronic fish data (figure 6). The lack of direct risk for fish is also assumed to occur for amphibians, as well. Uncertainty exists in the characterization of risk to amphibians as no methoxyfenozide toxicity data appears to be available; however, due to the very low risk to fish, and the conservative assumptions in the estimate of residues, amphibians would have to be a couple orders of magnitude more sensitive for risk to occur.

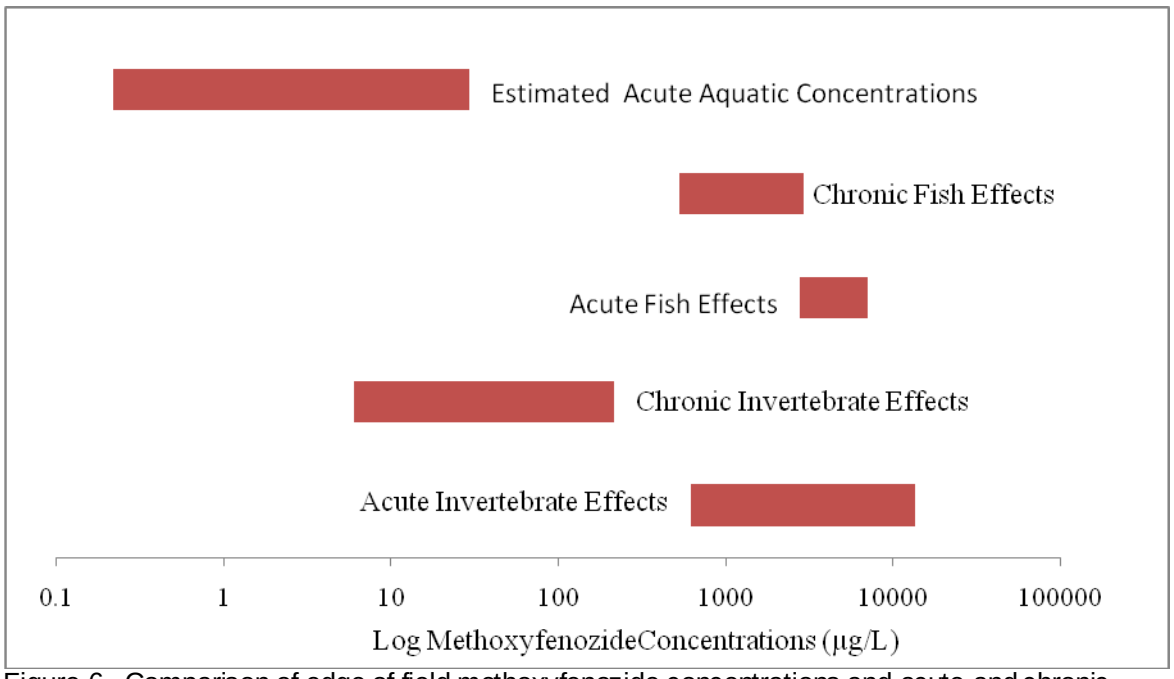

Figure 6. Comparison of edge of field methoxyfenozide concentrations and acute and chronic aquatic effects data.

Acute risk to aquatic invertebrates is also low with greater than an order of magnitude between the most sensitive aquatic invertebrate and the shallow waterbody habitat that was used in estimating methoxyfenozide residues. There is some overlap between acute methoxyfenozide residues in shallow aquatic habitats and the chronic invertebrate data. Label requirements are for a 25-foot no spray zone adjacent to a variety of aquatic habitats, including marshes and large reservoirs. The use of the application buffer reduces residues below the most sensitive chronic invertebrate effects value and will reduce the likelihood of impacts if multiple applications occur due to concerns about methoxyfenozide persistence.

Chlorantraniliprole

Chlorantraniliprole (Ryanaxypyr™) is a recently introduced insecticide that belongs to the anthranilic diamide insecticide class (figure 7). The mode of action is the activation of insect ryanidine receptors which causes an uncontrolled release of calcium from smooth and striated muscles which impairs muscle regulation and causes paralysis in insects (EPA, 2008b, Health Canada, 2008). Although these receptors occur in mammals, the insecticide is very selective to insect ryanidine receptors (Lahm et al., 2007).

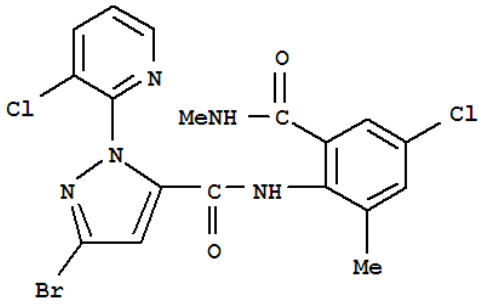

Figure 7. Chemical structure of chlorantraniliprole.

The formulation proposed for use in the EGVM program is Altacor®. Altacor® is registered for a variety of uses, but would be used in certain commercial applications to control EGVM and would be applied using ground application equipment.

Exposure Analysis

Chlorantraniliprole has low solubility in water with values ranging from 0.880 mg/L at pH 4, to approximately 0.972 at pH 7 and 9 (EPA, 2008b). Chlorantraniliprole is considered persistent in soil and water based on available laboratory and field degradation/dissipation studies (table 52). Chlorantraniliprole would not be expected to volatilize based on the reported low vapor pressure at variable temperatures (6.3 x 10-12 Pa @ 20 °C, 2.1 x 10-11 Pa @ 25 °C) (EPA, 2008b).

Table 52. Environmental Fate Parameters for Chlorantraniliprole.

Environmental Fate Parameter	Reported Half-life (days)
Hydrolysis	Stable @ pH 7
Aqueous Photolysis	0.31
Aerobic Soil Metabolism	228–924
Anaerobic Aquatic Metabolism	208
Aerobic Aquatic Metabolism	125–231
Terrestrial Field Dissipation	Up to 1,130

Terrestrial

Exposure to terrestrial vertebrates was estimated using the T-REX model similar to the previously discussed insecticides. Input parameters were based on maximum application rates and environmental fate that would result in higher residues from direct application of chlorantraniliprole to various food items (table 53). Maximum application rates and the minimal application intervals were selected from the Altacor® Section 3 and 2ee label for EGVM use.

Table 53. Input Parameters for T-REX Modeling.

Parameters	Chlorantraniliprole
Application rate (lb ai/ac)	0.099
Half-life (days)	924
Number of Applications	2
Application Interval (days)	7

ai = active ingredient

Using the above input parameters, the upper bound estimates of chlorantraniliprole of various food items was estimated with residues ranging from 2.96 ppm on large insect prey items to 47.40 ppm on short grass from direct application at the maximum application rate (table 54).

Table 54. Expected Upper Bound Chlorantraniliprole Residues (ppm) on Terrestrial Food Items Using T-REX.

Food Items	Chlorantraniliprole Residues (ppm)
Short grass	47.40
Tall grass	21.72
Broadleaf plants/small insects	26.66
Fruits/pods/seeds/large insects	2.96

The values listed above can be used to calculate exposure concentrations based on mg/kg body weight, and adjusted for different receptor classes using known ingestion rates.

Mammal body weights, ingestion rates, and percent of body weight consumed values are listed below for a representative herbivore, insectivore, and granivore mammal (table 55).

Table 55. Adjusted Mammal Parameters for Different Class and Body Size.

Mammalian Class	Body Weight (g)	Ingestion (dry) (g bwt/day)	Ingestion (wet) (g/day)	% Body Weight Consumed	Food Intake (kg-diet/day)
Herbivores/ Insectivores	15	3	14	95	1.43E-02
	35	5	23	66	2.31E-02
	1,000	31	153	15	1.53E-01
Granivores	15	3	3	21	3.18E-03
	35	5	5	15	5.13E-03
	1,000	31	34	3	3.40E-02

Using these values, estimated environmental concentrations (EECs) can be calculated based on the dose that each type of mammal would receive from different food items (table 56). This value can then be compared to the adjusted LD_{50} and NOEL values to determine if exposure levels exceed effect thresholds.

Table 56. Dose-based Chlorantraniliprole Estimated Environmental Concentrations for Mammals.

Dose-based EEC (mg/kg-bw)	Mammalian Classes and Body weight					
	Herbivores/Insectivores			Granivores		
	15 g	35 g	1000 g	15 g	35 g	1000 g
Short grass	45.19	31.23	7.24			
Tall grass	20.71	14.31	3.32			
Broadleaf plants/small insects	25.42	17.57	4.07			
Fruits/pods/seeds/large insects	2.82	1.95	0.45	0.63	0.43	0.10

Doses for birds can also be calculated based on body weight and consumption rates for a range of bird sizes (table 57). For this assessment, birds ranging in size from 20 to 1,000 g were used to calculate the amount of food that could be consumed in a given day.

Table 57. Adjusted Avian Parameters for Different Class and Body Size.

Avian Class	Body Weight (g)	Ingestion (Fdry) (g bw/day)	Ingestion (Fwet) (g/day)	% Body Weight Consumed	(kg-diet/day)
Small	20	5	23	114	2.28E-02
Mid	100	13	65	65	6.49E-02
Large	1000	58	291	29	2.91E-01

Dose-based EECs can also be made for different avian groups so that doses based on body weight and feeding preference can be compared to the adjusted toxicity effects endpoint (table 58). These comparisons are discussed in the risk characterization section of this risk assessment.

Table 58. Estimated Chlorantraniliprole Environmental Concentrations for Several Avian Classes.

Dose-based EEC (mg/kg-bw)	Avian Classes and Body Weights		
	small 20 g	mid 100 g	large 1000 g
Short grass	53.98	30.78	13.78
Tall grass	24.74	14.11	6.32
Broadleaf plants/small insects	30.36	17.31	7.75
Fruits/pods/large insects	3.37	1.92	0.86

Aquatic

Aquatic residues in shallow static habitats were low with edge of residues of 11.6 µg/L using the maximum application rate, broadcast applications, wind direction blowing towards the sensitive habitat, and no degradation or partitioning of the insecticide. The below residues represent 90th percentile residues that would be expected to occur under the modeled application scenario. The below residues do not account for potential runoff; however, applications directly to foliage and avoiding applications prior to rainfall events will reduce the potential for runoff as a major contributing factor to off-site aquatic habitats. Similar to the other insecticides discussed in this risk assessment, large reductions in drift occur with application buffers of 25 feet from the edge of aquatic habitats. Application buffers would also reduce the amount of runoff (table 59).

Table 59. Estimated Chlorantraniliprole Residues at Edge of Field and At Various Buffer Zones in a Shallow Closed Aquatic Hhabitat.

Chemical	Buffer Zone (ft)	Aquatic Residues (µg/L)	Percent Reduction in Residues
Chlorantraniliprole	0	11.6	-
	25	1.2	89.6
	50	0.7	94.0
	75	0.5	95.7
	100	0.4	96.6

Effects Analysis

Mammalian Toxicity

The technical material containing chlorantraniliprole is considered practically nontoxic via oral, dermal, and inhalation exposures (EPA, 2008b; DuPont, 2010). Comparisons with the formulated product suggest similar toxicity to the technical material. In a study where rats were dosed at concentrations ranging from 175 to 5,000 mg/kg, the results were no mortalities, clinical signs of toxicity, or body weight losses (table 60).

Table 60. Comparative Acute Mammalian Toxicity Between Technical and Formulated Chlorantraniliprole.

Test	Technical	Altacor®
Oral LD_{50}	>5,000 mg/kg	>5,000 mg/kg
Dermal LD_{50}	>5,000 mg/kg	>5,000 mg/kg
Inhalation LC_{50}	>5.1 mg/L	>6.2 mg/L

The formulation proposed for use in this program is not considered an irritant to the eyes or skin, and is not a skin sensitizer. In addition, the technical material is not considered to be carcinogenic or mutagenic, and is not known to cause reproductive or developmental toxicity. The NOEL value in reproductive and developmental toxicity studies was 1,000 mg/kg/day, or the highest concentration tested (EPA, 2008b) (table 61). Studies designed to assess

neurotoxicity and effects on the immune system show no effects at a range of doses from the low mg/kg range to greater than 1,000 mg/kg.

Table 61. Subchronic and Chronic Mammalian Toxicity Values for Chlorantraniliprole.

Toxicity Test/ Test Species	NOEL (mg/kg/day)
Subchronic Neurotoxicity/Rat	1,313 males / 1,586 females
Subchronic 28-day Immunotoxicity/Rat	1,494 males / 1,601 females
Subchronic 28-day Immunotoxicity/Mouse	1,144 males / 1,516 females
Chronic 1-yr/Dog	1,164 males / 1,233 females
Chronic/Carcinogenicity 2-yr/Rat	885 males / 1,076 females
Carcinogenicity 18-month/Mouse	158 males / 1,155 females
Developmental Toxicity/Rat	1,000
Developmental Toxicity/Rabbit	1,000

The lowest reported LD_{50} and NOEL from the developmental and reproductive studies (1,000 mg/kg) were used to estimate adjusted effect values for a range of mammals based on their body weight and the percentage of their body weight consumed. The ranges selected were designed to represent mammals, such as the shrew, that consume a large percentage of their body weight and may receive higher exposure, as well as larger mammals (1 kg) that may consume less when compared to the standard laboratory rat which is used in several of the mammalian toxicity studies (table 62). The acute and chronic toxicity values are considered conservative because no effects were seen at the reported concentrations, actual effects would be seen at higher doses and, therefore, result in higher adjusted effect values.

Table 62. Adjusted LD_{50} and NOEL Values for Select Mammals.

Mammalian Class	Body Weight	% Body Weight Consumed	Adjusted LD_{50}	Adjusted NOEL
Herbivores/ Insectivores	15	95	10,989.15	2197.83
	35	66	8,891.40	1,778.28
	1,000	15	3,845.80	769.16
Granivores	15	21	10,989.15	2197.83
	35	15	8,891.40	1,778.28
	1,000	3	3,845.80	769.16

Avian and Reptile Toxicity

The acute toxicity of chlorantraniliprole to birds is very low with no acute lethal or sublethal effects noted at all doses in the oral gavage or dietary studies (table 63). Chronic toxicity was also low in 22-week exposure studies used to evaluate reproductive impacts. The NOEC was reported as 120 and 250 ppm, respectively, for the bobwhite quail and mallard.

Table 63. Toxicity of Chlorantraniliprole to Select Avian Species.

Test Species/Duration	LD_{50}/LC_{50} (mg/kg)	NOEL/LOEL (mg/kg)
Bobwhite quail LD_{50}	>2250	2250/NR
Bobwhite quail LC_{50}	>5620	5620/NR
Bobwhite quail chronic reproduction	NR	120/520
Mallard LC_{50}	>5620	5620/NR
Mallard chronic reproduction	NR	250/500

NR = Not reported

The lowest acute NOEL value was selected (2,250 mg/kg) to estimate a range of sensitivities to birds based on different body weights and food consumption (table 64).

Table 64. Adjusted Acute Toxicity Values for Different Sized Avian Receptors.

Avian Class	Body Weight (g)	% Body Weight Consumed	Adjusted NOEL
Small	20	114	1,620.97
Mid	100	65	2,063.57
Large	1,000	29	2,914.87

The range of adjusted NOEL values can then be compared to the exposure values for the different food items and range of bird sizes to better characterize risk to a variety of birds species that could be exposed to treated food items if they were to forage in areas that were directly treated with chlorantraniliprole.

A review of the literature and available databases suggests that no reptile toxicity data appears to be available for chlorantraniliprole. As stated previously in those cases where reptile toxicity data is not available, the avian data has been used as a surrogate to characterize sensitivity to reptiles. Chlorantraniliprole would be expected to be practically nontoxic to reptiles based on the available avian toxicity data.

Terrestrial Invertebrate Toxicity

Available toxicity data for technical and formulated chlorantraniliprole to honey bees shows low toxicity from oral and contact exposure, with effect values greater than 100 µg/bee or above solubility (EPA, 2008b). Semi-field studies with the formulated material has also demonstrated low residual toxicity with a NOEC of 60 g/ha. Toxicity studies testing other terrestrial invertebrates, such as earthworms, report low acute and chronic toxicity when exposed to the technical and formulated material, as well as primary metabolites (EPA, 2008b). Other terrestrial invertebrates, such as the springtails and some beneficial invertebrates, appear to have low sensitivity to chlorantraniliprole (EPA, 2008b).

Terrestrial Plant Toxicity

Available terrestrial phytotoxicity data for a 20SC formulation of chlorantraniliprole shows very low toxicity, with reported EC_{25} values greater than 300 g/ha for several monocot and dicot plant species (EPA, 2008b). This is approximately three times the proposed maximum application rate in this program. Exposures occurred to seeds in one set of studies to evaluate emergence, while small developing plants were exposed in another study.

Aquatic Toxicity

Chlorantraniliprole toxicity to fish is considered low based on available toxicity data that reports lethality occurring above solubility. Longer term exposures show that sublethal impacts may occur at concentrations exceeding 0.11 mg/L (table 65) (EPA, 2010).

Table 65. Acute and Chronic Toxicity of Chlorantraniliprole to Fish.

Test Species/ Duration	LC_{50}/EC_{50} (mg/L)	NOEC/LOEC (mg/L)
Acute Tests		
96-hour LC_{50} Bluegill Sunfish	>15.1	NR/NR
96-hour LC_{50} Rainbow Trout	>13.8	NR/NR
96-hour LC_{50} Sheepshead Minnow	>12.0	12.0/NR
Subchronic Tests		
Rainbow Trout 31-d ELS (early life stage)	NR	0.11/NR
Sheepshead 35-d ELS	NR	1.28/NR

*ELS = Early life stage study; NR = Not reported

Aquatic invertebrates are more sensitive to chlorantraniliprole acute exposure compared to fish with values ranging from 0.0098 mg/L for the cladoceran, *D. magna* to 1.15 mg/L for marine mysid shrimp (EPA, 2010; Barbee et al, 2010). Available median lethality data for several insect species shows values ranging from 0.116 mg/L to greater than 0.978 mg/L (table 66).

Table 66. Acute Toxicity of Technical Chlorantraniliprole to Aquatic Invertebrates.

Test Duration/Species	LC_{50}/EC_{50} (mg/L)	NOEC (mg/L)
Acute Tests		
48-hour EC_{50} *Daphnia magna*	0.0098	NR
48-hour EC_{50} *Daphnia magna*	0.0116	NR
48-hour LC_{50} *Lumbriculus variegatus* (aquatic annelid)		
48-hour LC_{50} *Brachionus calyciflorus* (rotifer)	>1.00	NR
48-hour LC_{50} *Hyallela azteca* (amphipod)	>0.389	NR
48-hour LC_{50} *Gammarus pseudolimaeus* (amphipod)	0.0351	NR
48-hour LC_{50} *Chimarra atterima* (caddisfly)	0.0117	NR
48-hour LC_{50} *Centroptilum triangulifera* (mayfly)	0.0116	NR
48-hour LC_{50} *C. riparius* (midge)	0.0859	NR
48-hour LC_{50} *Soyedina carolinensis* (stonefly)	>0.978	NR
96-hour LC_{50} *Procambarus clarkii*	0.951	0.480
96-hour LC_{50} Mysid Shrimp	1.15	NR
96-hour LC_{50} Eastern Oyster	0.0399	NR
Chronic Tests		
21-day *Daphnia magna* (life cycle)	NR	0.0045
28-day Mysid Shrimp (life cycle)	NR	0.695

NR = Not reported

Acute aquatic toxicity data for the formulated product is comparable to the range of sensitivities that have been reported for the technical material (table 67). The proposed formulation contains 35 percent active ingredient with the remaining other ingredients composing 65 percent of the water dispersable granular formulation. Identification of the ingredients is considered confidential business information and is not stated on the current material safety data sheet.

Table 67. Aquatic Toxicity of Formulated Chlorantraniliprole to Aquatic Organisms.

Test Duration/Species	LC_{50}/EC_{50} (mg/L)	NOEC (mg/L)
48-hour EC_{50} *Daphnia magna*	0.0110	NR
96-hour LC_{50} Bluegill	>1.19	NR
96-hour LC_{50} Rainbow Trout	>1.19	NR
72-hour EC_{50} *Selenastrum capricornutum*	>1.78	1.78

NR = Not reported

Available aquatic plant toxicity data suggests low toxicity of chlorantraniliprole to freshwater and marine diatoms and algae, as well as aquatic macrophytes. Estimated EC_{50} and NOEC values were all greater than the highest test concentration used in the studies which ranged from 1.78 to 15.1 mg/L (EPA, 2008b).

Risk Characterization

Terrestrial (Mammals, Birds and Reptiles)

Comparing the upper bound estimates of residues that could occur from direct applications of chlorantraniliprole to various food items to the more sensitive toxicity endpoints shows that the risks to various mammals is extremely low in acute and chronic exposures. The RQ values were typically less than 0.01 and, in this case, are actually lower because the effect values selected were the highest test concentration and no adverse effects were noted (table 68). These estimates of risk are also conservative because they assume each mammal group would feed exclusively on treated food items in an area treated with the maximum application rate of insecticide.

Table 68. Dose-based RQ Values for Multiple Mammal Classes.

Dose-based RQs (Dose-based EEC/LD50 or NOEL)	15 g mammal		35 g mammal		1000 g mammal	
	Acute	Chronic	Acute	Chronic	Acute	Chronic
	<0.01	0.02	<0.01	0.02	<0.01	0.01
Short grass	<0.01	0.01	<0.01	0.01	<0.01	<0.01
Tall grass	<0.01	0.01	<0.01	0.01	<0.01	0.01
Broadleaf plants/small insects	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Fruits/pods/large insects	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Seeds (granivore)	<0.01	0.02	<0.01	0.02	<0.01	0.01

Acute direct risk to avian species is also considered low with exposure levels well below the acute NOEL values that were estimated for each bird and food item consumed (table 69). Risk would actually be much less because the NOEL was the highest test concentration tested in the LD_{50} study.

Table 69. Dose-based RQ Values for Multiple Avian Classes.

Dose-based RQs (Dose-based EEC/NOEL)	Avian Acute RQs		
	20 g	100 g	1000 g
Short grass	0.03	0.01	<0.01
Tall grass	0.02	0.01	<0.01
Broadleaf plants/small insects	0.02	0.01	<0.01
Fruits/pods/large insects	<0.01	<0.01	<0.01

Chronic risk to birds is also considered low. A comparison of the lowest reported NOEC value from the bobwhite quail reproductive study (120 ppm) to the range of upper bound estimates of exposure concentrations on various food items (2.96–47.40 ppm) demonstrated RQ values ranging from 0.02 to 0.39.

Acute and chronic risk to reptiles was considered comparable to estimates for birds. Uncertainty regarding this assumption is based on the lack of toxicity data for reptiles; however, the very low toxicity and risk to both mammals and birds would suggest that reptiles would also be protected. Sensitivity of reptiles to chlorantraniliprole would have to be at least two orders of magnitude higher for potential risk based on conservative assumptions regarding exposure.

Indirect risks to terrestrial vertebrate populations from the loss of terrestrial invertebrates that serve as a food source would not be expected due to the lack of broad spectrum activity of chlorantraniliprole to insects and the methods of application proposed for this use. Ground based applications will reduce potential impacts to terrestrial invertebrates to areas in, and immediately adjacent to, application sites. Label requirements for drift management will also reduce the potential for off-site transport. Although impacts to Lepidoptera are expected in treated areas, these areas are not expected to be larger than the foraging range for most terrestrial vertebrates, and other invertebrates would be available that are not sensitive to chlorantraniliprole.

Terrestrial vertebrates that depend on aquatic invertebrates and fish for prey would also be expected to be at low risk from the potential loss of prey. Risk is expected to be low to aquatic biota (discussed below). Ingestion of contaminated prey is also expected to be a minor pathway of exposure as chlorantraniliprole is not expected to bioconcentrate with a reported bioconcentration factor value of <21 (EPA, 2008b).

Terrestrial (Plants and Invertebrates)

Direct risks to terrestrial plants are not expected because chlorantraniliprole is not considered phytotoxic, based on available data. Indirect risk to terrestrial plants that are insect-pollinated is also expected to be low in most cases due to the lack of broad spectrum activity against insect pollinators other than Lepidoptera. Available terrestrial invertebrate toxicity data, with the exception of Lepidoptera, show low toxicity to other potential pollinators. In the case of rare and listed plants, an application buffer may be required if applications are expected to occur during pollination, and Lepidoptera are important in pollinating those plants.

Aquatic

A comparison of the range acute aquatic concentrations to the available acute and chronic fish data suggest minimal direct risk to fish from exposure to chlorantraniliprole. Acute risk would actually be less because lethal and sublethal responses were higher than the highest test concentration for all test species. Direct risk to other aquatic vertebrates, such as amphibians, would also be expected to be low. Uncertainty exists in the characterization of risk to amphibians because no data appears to be available; however, based on the lack of direct risk to fish well above solubility limits for chlorantraniliprole, and the conservative assumptions in the estimate of residues, amphibians would have to be several orders of magnitude more sensitive for direct risk to occur.

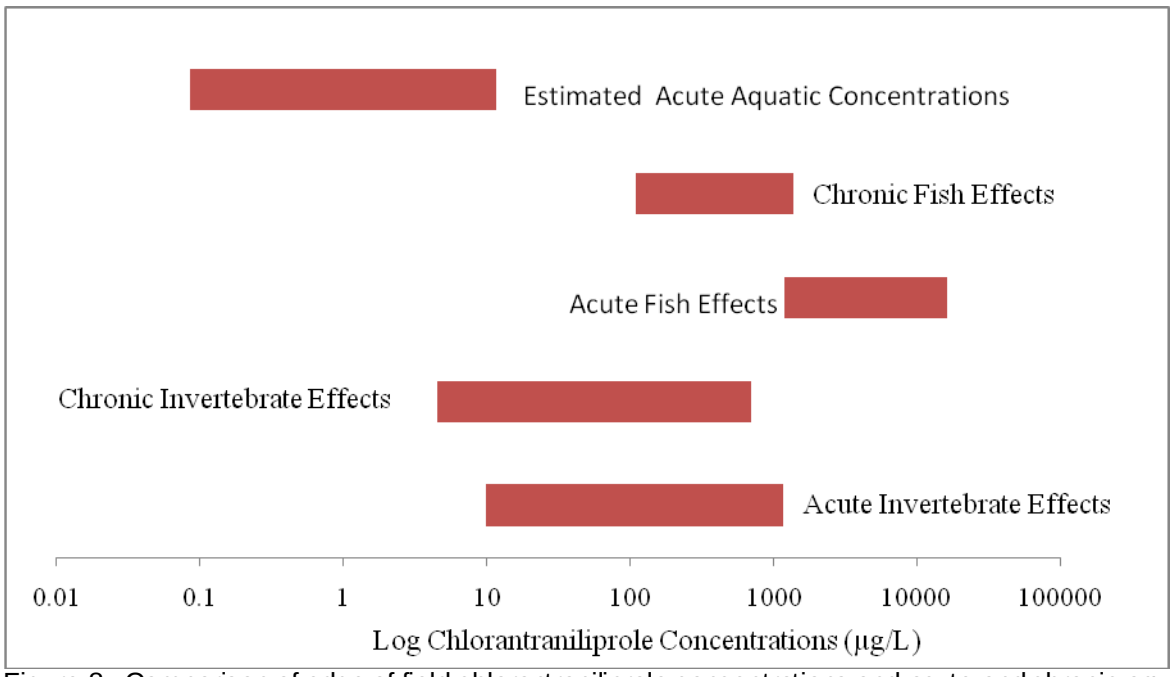

Figure 8. Comparison of edge of field chlorantraniliprole concentrations and acute and chronic aquatic effects data.

Indirect risk to fish and amphibians can also occur through the loss of prey or habitat. Chlorantraniliprole exhibits low toxicity to aquatic plants and would not be expected to impact aquatic vertebrate habitat or reduce the amount of algae that would be used to support aquatic invertebrates that could serve as prey for fish and amphibians. Acute risk to aquatic invertebrates is not expected because the range of effects data is greater than an order of magnitude above the range of expected concentrations. There is some overlap in the chronic aquatic invertebrate effects data and the residues that could occur in shallow enclosed waterbodies. This comparison is conservative as it takes acute concentrations and compares to chronic exposure data which in this case ranges from 21 to 37 days, depending on the study. A more appropriate comparison would be to consider degradation/dissipation under more realistic application scenarios, which would demonstrate lower potential residues. Chlorantraniliprole is resistant to degradation in water; however, it does have low solubility and can partition to sediment which would reduce bioavailability to water column invertebrates. In addition, label language regarding the use of vegetative filter buffers between treatment areas and aquatic habitat will reduce the amount of potential off-site transport from chlorantraniliprole applications.

Summary

This screening level environmental risk assessment evaluated the potential direct and indirect risks of five products that are proposed for use in the control of EGVM in noncommercial and/or commercial nursery applications. The pheromone may be used in conjunction with one of four foliar applied insecticides to ensure effective control of EGVM in areas immediately adjacent to trap detections, or in nursery applications, where labeled for use. Three of the five products discussed in this assessment can also be used in organic applications. These treatments will only be used if fruit/flower or host plant removal is not possible. Available effects and environmental

fate data, along with conservative estimates of exposure, suggests that direct and indirect risk of all program applications under the described uses will not have detrimental impacts to terrestrial and aquatic vertebrate populations. Insecticides proposed for foliar applications may impact some nontarget terrestrial invertebrates in the area of application; however, these impacts will be variable based on the specific chemistry. None of the foliar applied insecticides are considered broad spectrum which reduces their impacts to nontarget invertebrates, and each are considered products that can be used in integrated pest management plans.

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