2020 Review of Biological Pesticides for Treatment of Browntail Moth Near Marine Waters

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Executive Summary

This risk assessment evaluates a suite of pesticide active ingredients used during treatment of browntail moth infestations when applied from 25 to 50 feet from the high-tide line with powered equipment.

The active ingredients assessed to have acceptable risk for this use near marine habitats include:

Bacillus thuringiensis subspecies *kurstaki* & *aizawai*
Beauveria bassiana
GS-omega/kappa-Hxtx-Hv1a
Isaria fumosorosea
Kaolin Clay
Spinosad

Method
Based on selection criteria provided by the Maine Forest Service, pesticides with labeled uses for gypsy moths were queried in a database containing pesticides registered in Maine for 2020. Of those pesticides, candidates for the biological pesticides list were searched by use site and chemical categorization (biochemical and microbial pesticides were selected). Eight candidate active ingredients were identified for this risk assessment.

For each active ingredient, data were collected on environmental fate and transfer parameters along with toxicity data for marine and estuarine organisms. When insufficient data were available for quantitative risk assessment approaches, available data were summarized qualitatively. Risk was assessed by comparing expected environmental concentrations (EEC) to the concentrations known to produce toxic effects in marine and estuarine organisms.

Outcome
Seven of the eight candidate active ingredients were deemed unlikely to cause undue harm to marine and estuarine organisms. The only candidate chemical that was not selected for the current list was azadirachtin. Azadirachtin is a biochemical extracted from neem seeds with high toxicity to aquatic organisms. Each of the remaining active ingredients were deemed unlikely to cause undue harm to marine and estuarine organisms when products were used as labelled.

This revised list includes two subspecies of one bacterium (*Bacillus thuringiensis*), two fungi (*Beauveria bassiana* and *Isaria fumosorosea*), one product of bacterial synthesis not including live bacterial spores (spinosad), one biochemical mechanical disruptor (kaolin clay), and one protein toxin that originates from spider venom (GS-omega/kappa-Hxtx-Hv1a). These additional pesticide options allow for more flexibility in the treatment strategies and resistance management.

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Introduction

In 2016, the Maine Board of Pesticides Control (BPC) established that only "biological pesticides" were allowed for use in the zone of land 25 to 50 feet from the high-tide line during the treatment of browntail moth, including with powered application equipment. In 2017, the BPC clarified the meaning of biological pesticides with a policy that listed the active ingredients that met the definition of biological pesticide and was suitable for applications to treat browntail moths. The BPC clarification indicates that a biological pesticide is comprised of either 1) microbiological organisms or 2) products produced by and commonly associated with organisms. This document re-evaluates and provides updated suggestions for those pesticide active ingredients in the biological pesticides category for the purposes of Chapter 29 Section 5.

The rationale for this designation stems from general patterns that are frequently seen in biological pesticides. Biological pesticides often have modes of action that are targeted to a more specific group of pests. Also, some biological pesticides have a short residence time in the environment. The co-location of browntail moths and coastal habitat has warranted that the pesticides used as close as 25 feet from the ocean to represent the lowest risk products available.

This risk assessment document concerns the treatment of browntail moths in the area 25 to 50 feet from the marine coast. Pesticides allowed in the biological pesticides category are varied in their mechanism of action. This is a broad category and as such each pesticide in this group has required a unique approach to its risk assessment. Biochemical pesticides are naturally occurring substances with a pesticidal nature. The previous list of "biological pesticides" included azadirachtin and spinosad both of which are classified by US EPA as biochemical pesticides. Other examples of biochemical pesticides would include: kaolin clay, GS omega/kappa-Hxtx-Hv1a, and smothering oils like horticultural oil and caustic ingredients such as horticultural vinegar. Microbial pesticides, on the other hand, are comprised of the living organism itself. Examples of microbial pesticides include *Bacillus thuringiensis* subspecies *kurstaki* & *aizawai, Beauveria bassiana,* and *Isaria fumosorosea.*

This risk assessment evaluates the hazard of each of the proposed biological pesticides to understand the potential for exposure to marine organisms and then assess whether the risk from their use is acceptable in the marine environment.

Method

Following guidance from the Maine Forest Service (MFS), identification of the pesticides to be reviewed started with a search for active ingredients effective against gypsy moths. Gypsy moths are used as a surrogate pest species because there is a lack of research and knowledge on effective pesticide approaches to browntail moth control. Pesticides with labeled uses for gypsy moths were queried in the National Pesticide Information Retrieval System database containing pesticides registered in Maine for 2020. Of those pesticides, candidates for the biological pesticides list were searched by use site and chemical categorization (biochemical and microbial pesticides were selected). Eight active ingredients were identified for this risk assessment.

For each active ingredient, data were collected on environmental fate and transfer parameters along with toxicity data for marine and estuarine organisms. Available data were summarized qualitatively when insufficient data were available for quantitative risk assessment approaches. Risk was assessed by comparing

expected environmental concentrations (EEC) to the concentrations known to produce toxic effects in marine and estuarine organisms.

Specific detail of the risk assessment methods are found in the sections for each candidate active ingredient. Each of these biopesticides are unique and vary in the types of assessment data available.

Azadirachtin

Azadirachtin is a pesticidal extract taken from the seeds of the neem tree, *Azadirachta indica A Juss.*. In a technical sense "azadirachtin" is a term that represents a loosely described collection of chemicals; this group of active compounds is characterized by azadirachtin A, one of the most abundant compounds in the group. Azadirachtin is considered to be different from cold-pressed neem oil because there are more compounds in cold-pressed neem oil than in solvent extracted azadirachtin.

Azadirachtin has multiple effects. In some insects azadirachtin has been shown to be a repellant and feed inhibitor. It is also an insect growth regulator. Azadirachtin is understood to block the insect hormone ecdysone and it is lethal to insects because they cannot metamorphosize without proper ecdysone levels. It must be consumed to be effective.

Toxicity

Azadirachtin is practically non-toxic to mammals and birds. In acute mammalian testing, azadirachtin had low toxicity (Category III) for both oral and dermal exposures and very low toxicity (Category IV) for inhalation and dermal irritation exposures. Due to the practically non-toxic profile US EPA did not require further testing or calculate a quantitative dietary and drinking water toxicity assessments.

Azadirachtin has moderate to high toxicity to aquatic organisms. There are no marine or estuarine data for azadirachtin, this risk assessment substituted freshwater data. In freshwater fish, exposures as low as 0.0047 ppm were found to potentially cause effects. Freshwater invertebrates varied in their sensitivity: the No Observed Adverse Effect Concentration (NOAEC) for water fleas, *Daphnia* species, was 0.615 ppm while the NOAEC for midges, *Chironomus* species, was 0.0016 ppm representing a difference over 350 times lower than the water flea.

Azadirachtin has an exemption for all raw agricultural commodities from the required tolerance when used at a rate of less than 20 grams per acre.

Environmental Exposure

Exposures to aquatic organisms are predicted to be low when the product's label instructions are followed. As a seed extract, this compound is more oily than watery in nature and is not highly mobile in soil. It breaks down quickly in sunlight on the foliage and on soil. The half-lives are measured in hours and days and it is expected to be half degraded in less than a day to two days. Once in the soil, it is rapidly consumed by soil organisms. The aquatic half-life is longer and considered to be around 30 days or less.

A quantitative risk assessment was possible with azadirachtin because substantial fate, transfer, and toxicity data are available. US EPA's Pesticide in Water Calculator Version 1.52 was used to predict the Expected Environmental Concentration (EEC) under the standardized pond scenario used by US EPA. The modeled peak concentration was 0.0013 ppm following the predicted drift and runoff from an application to a modeled apple orchard with air blast sprayers. The modeled 21-day average EEC was 0.0007 ppm.

The calculated Risk Quotient (RQ) for an acute exposure was 0.85; any RQ value higher than 0.5 demonstrates unacceptable risk to aquatic organisms. For chronic exposures an RQ value less than 1 indicates acceptable risk. The calculated chronic RQ for azadirachtin was 0.16 indicating acceptable risk from the modeled use.

Conclusion

The biochemical azadirachtin did not pass the risk assessment standards used to ensure there is no undue harm caused by lawful uses. The acute toxicity of azadirachtin was too high given the environmental concentrations predicted by the model following labeled uses. This active ingredient has not been included on the updated list of biological pesticides allowed within 25 to 50 feet of the high-tide line. A brief review of details of lobster and clam physiology confirms that ecdysone plays important roles in their normal development leaving open the possibility of azadirachtin directly affecting these organisms.

Bacillus thuringiensis subspecies *kurstaki* & subspecies *aizawai*

Bacillus thuringiensis (*Bt*) is a widely used bacterial insecticide cultured from a soil bacterium. Different *Bt* subspecies and strains can affect different types of target pests including: Diptera, Coleoptera, Lepidoptera, Hemiptera, and Hymenoptera. On the whole "*Bt*" targets a range of organisms but in practice each subspecies and strain have limited selectivity. In this review, *Bt* is a term used to refer to all the species and strains of *B. thuringiensis* subspecies *kurstaki* and *B. thuringiensis* subspecies *aizawai* as a single group not because there are no important differences, but because as of to date none of the differences between subspecies *kurstaki* and *aizawai* are substantially dissimilar for the context of this risk assessment which focuses on risk to aquatic non-target organisms.

The mode of action for *Bt* is sometimes disputed but generally understood as follows. The primary toxins produced by the *Bt* bacteria, δ(delta)-endospores, are only available after being activated by proteins in an insect's midgut. Additionally, the pH must be alkaline for this activation to take place. The activated toxins interact with the cells of the intestines and cause those cells to die. A combination of intestinal trauma and secondary infection (once the intestinal lining is breached bacteria move throughout the body) contribute to the organism's death over the course of a few days. Multiple risk reviews have demonstrated that *Bt* is not harmful to most organisms because the conditions are not right for the primary toxins to become available and active.

Bt subspecies *kurstaki* and *aizawai* are in the same family as *B. cereus* and *B. anthracis*. Contamination of the fermentation vats used in production of *Bt* with these similar taxa is a human health concern. Each batch of the product is tested in a live mouse assay and each change in formulation processing requires another round of assays demonstrating no additional genetic material has been added.

Toxicity

In addition to the δ-endospores, *Bt* subspecies produce scores of toxins which contribute to the specificity of *Bt*'s toxicity in different subspecies and strains. The complete complement of toxins for *Bt* organisms contains: the primary toxins (δ-endotoxins) Cry and Cyto; and parasporins, Vips, Sips, Bins, 41.9-kDa protein, sphaericolysins, alveolysins, β-exotoxins (like thuringiensin), enhancin-like proteins, and P19/P20 helper proteins (Palma et al. 2014). The value of *Bt*'s selectivity stems from variations in the toxins produced. Toxicity of each *Bt* product is unique and determined by the mixture of: species and age of the pest, subspecies of *Bt* bacteria, strain of the *Bt* subspecies, the concentration of active ingredients (the crystal and cytolytic proteins, the δ-endotoxins), the concentration of exotoxins and enterotoxins, inert ingredients of the formulation, and concentration of spores.

Non-target effects likely originate from these additional toxins that do not require a specific gut pH, however, these other toxins are currently not present at locations and quantities to cause significant toxicity.

While the specifics for each possible combination are not understood, 50 years of use has produced patterns that demonstrate very clearly a lack of vertebrate toxicity, only very minimal toxicity to non-target organisms, and expected toxicity to target organisms. *Bt* was found to be practically nontoxic to grass shrimp, sheepshead minnow, and copepods during standardized testing. Only one study out of many reviewed found negative effects from *Bt kurstaki*, in that study stonefly larvae increased their drift behavior when exposed to ten times the Expected Environmental Concentration (EEC) (USFS 2007). Other studies show aquatic invertebrates able to

withstand exposures 200,000 times the EEC. With the fish species tested, exposures have caused harm to fish when the test substance volume increased to a level that caused oxygen depletion and the fish suffocated.

Environmental Exposure

The persistence in the environment of *Bt* is generally thought to be short. Sunlight is a strong agent of breakdown and foliar half-lives are on the order of 2 to 3 days. Under better conditions *Bt* may remain viable for 4 to 5 days on the leaf surface. In soil, *Bt* spores can persist for weeks, spores are largely destroyed by sunlight and soil organisms. *Bt*'s ability to re-infect is considered to be poor, so the likelihood of those spores persisting beyond that point is very low. The persistence of the δ-endotoxins is longer than the spores and it has been observed that they can be detected for around a month.

Conclusion

A review of the toxicity data indicated no patterns of toxicity to non-target aquatic organisms, from the products that target Lepidopterans. This review combines *Bt kurstaki* and *aizawai* and treats them as equivalent, this review specifically does not include *Bt israelensis*. *Bt israelensis* is frequently used in treatment of aquatic pests and clearly poses different risks to aquatic environments.

Beauveria bassiana

Beauveria bassiana is a naturally occurring soil fungus used to control a variety of insects, such as: aphids, apple clearwing moth, codling moth, Douglas fir tussock moth, European corn borer, silkworms, thrips, and termites. *Beauveria bassiana* can be a highly efficient lethal agent to insects from contact exposure and does not need to be ingested to work. Its mode of action is to grow and feed on the insect's body, this leads to softening of the exoskeleton and destruction of inner tissues.

Toxicity

Toxicity of *B. bassiana*, like all substances is highly dependent on concentration. *B. bassiana* is used in entomovectoring systems; in entomovectoring, insects like bumblebees, are used to disperse pesticide instead of the typical sprayer or irrigation technologies. The fact that *B. bassiana* can be used with bumblebees illustrates the importance of concentration in risk assessment as *B. bassiana* is also considered to be toxic to bees.

While the mode of action is infecting target organisms, fungi are capable of producing toxins that can also have toxic effects. The risks from unintentional toxin production (mycotoxins) is low. Beauvaricine is a known contaminant that can occur during the production of *B. bassiana* pesticide formulations. Changes to manufacturing and testing keep this toxin at low levels and below the level of concern.

Several studies have found that *B. bassiana* has toxicity to aquatic organisms. In a study with *Culex pipiens* Hamid et al. (2013) found high toxicity to exposed eggs and larvae. The method of exposure is noteworthy with respect to applicability to this risk assessment, eggs and larvae were dunked in a test solution containing *B. bassiana* and then returned to their home lake water. This study found complete mortality at a concentration of 0.33×10^{10} spores/L (higher that the Expected Environmental Concentration (EEC) for this product when used as labeled). An LC_{50} of 7,300 ppm was established for Rainbow Trout (*Oncorhynchus mykiss*), classifying *B. bassiana* as practically non-toxic to fish. When Inland Silversides (*Menidia berylinna*) embryos were exposed to *B. bassiana* at 8.3×10^4 to 1.5×10^5 conidia/mL the embryos had increased rate of rupture and death. These authors also tested the role of detergent-treated spores and concluded that the detergent prevented conidia from sticking to the embryos and lowered the mortality to the embryos.

In terrestrial invertebrates there were instances where the predicted environmental concentration would be expected to produce toxic effects. This is expected when evaluating an insecticide intended for terrestrial invertebrates. Earthworms showed no effects at concentrations as high as 1,000 ppm.

In other organisms, testing has produced no signs of toxicity beyond slight dermal irritation in rats at five times the labeled application rate. In mammals, there were no effects seen at doses produced by legal application. More specifically in rats, an oral dose of 1.9×10^8 ppm cleared within 3 days with no toxic effects and similarly high doses were cleared following inhalation and injection to the abdominal cavity. Injection into the abdominal cavity reinforces that this organism is not likely to be pathogenic to mammals. In birds, there were no effects seen at doses produced by legal application.

This review considers the strains of *B. bassiana* together as one unit. However, one of the available strains does not have an established tolerance, there are no food uses registered for *B. bassiana* Strain 447 (PC Code 128815). *Beauveria bassiana* strains: ATCC 74040 (PC Code 128818), GHA (PC Code 128924) and HF23 (PC Code

090305) are exempted, with qualifications and uses, from the requirement of a tolerance (40 CFR 180.1205, 40 CFR 180.1146, and 40 CFR 180.1273 respectively).

Environmental Exposure

This fungus is considered to be worldwide in distribution and widespread in the soil. It is not common in water sampling; one survey found that only 2% of their freshwater samples contained evidence of *B. bassiana*. Label mixing instructions indicate the product dies within 24 hours of mixing with water. *Beauveria bassiana* is classified as persistent in soil. Testing demonstrated that environmental concentrations declined to almost normal background levels in 6 months to 1 ½ years. This property is considered a benefit as it means areas can be treated in a way that inoculates against future pest outbreaks. While persistent *B. bassiana* does not significantly amplify in the environment or bioaccumulate; in this case, the higher background levels plateau at levels above background but below levels considered to cause harm.

It is difficult to model the expected environmental concentrations of biological pesticides largely because the chemical parameters typically used in modeling simply do not apply to pesticides like these - especially living organisms like *B. bassiana*. In lieu of standard modeling, the alternative approach in aquatic systems is to predict the aquatic concentration as though the application was made directly to the water using the labeled application rate. This is a highly conservative approach that essentially represents 100% drift from the target site to the waterbody. Using this method the expected environmental concentration (EEC) is 0.037 ppm also expressed as 3.7×10^6 conidia/L. Even with a conservative approach to estimating the EEC, the potential harm to non-target organisms is considered to be low. Fish showed effects over 100,000 times higher than the EEC. Mosquito larvae effects were seen at a brief exposure 10,000 times the EEC. And representing marine and estuarine organisms, silversides reacted to concentrations 1,000 times higher than the EEC. The risks stemming from use of *B. bassiana* focus on incidentally exposed terrestrial invertebrates and not aquatic organisms.

Conclusion

While much remains to be specifically determined about the potential for *B. bassiana* to persist and be effective in marine environments, *B. bassiana* is not expected to cause non-target harm when used as labeled. *Beauveria bassiana* is not expected to persist long in aquatic environments reducing the potential for exposure with marine and estuarine organisms. If terrestrial invertebrates are exposed to labeled dose rates mortality can be expected. However, harmful exposures to aquatic habitats are not expected from labeled uses due to the dilution of active ingredient that occurs during use.

GS-omega/kappa-Hxtx-Hv1a

GS-omega/kappa-Hxtx-Hv1a is a relatively new insecticide. This compound has a variety of names including Versitude™ peptide; GS-UACTX-Hv1a-SEQ2; GS-U-ACTX-Hv1a-SEQ2; M-ACTX-HV1a+2; "the spider venom pesticide"; and its brand names of Spear, Spear T, VST-006325 TGAI, & VST-006330 EP. Currently, GS-omega/kappa-Hxtx-Hv1a is the compound's active ingredient name as listed on current pesticide labels. This pesticide is manufactured by yeast using a gene sequence that produces a toxic peptide protein chain. In nature, this peptide is part of the venom used by Australian Blue Mountains Funnel Web Spiders (*Hadronyche versuta*) to kill their prey. It effects insects by depressing the central nervous system, specifically its action is to inhibit voltage-gated calcium and potassium channels highly specific to insect nervous systems.

Browntail moth is listed as a species on the label for GS-omega/kappa-Hxtx-Hv1a. This product currently contains 2% GS-omega/kappa-Hxtx-Hv1a and is marketed towards treatment of lepidopterans. The low risk nature of this insecticide stems from the targeted nature of the peptide, only organisms similar to the spider's natural diet in the wild appear to be affected, such as Coleoptera, Lepidoptera, and Thysanoptera.

Toxicity

The active ingredient is produced by fermentation of yeast which are removed from the product as part of manufacture. The yeast species involved, *Kluyveromyces lactis*, is used in the manufacture of foods; it and any by-products are not anticipated to cause harm to humans. Cell culture studies have shown no effect on mammalian cells.

Acute toxicity testing on mammals, birds, fishes, and aquatic invertebrates demonstrated in all cases there was no mortality at the maximum dose that is feasible to administer. For bees, there was a contact LD_{50} value lower than the maximum feasible dose, however, it is still expected to have practically no toxicity to bees. Oral exposure in bees produced no observable effects at the highest dose feasible. Additionally, no sublethal effects were seen during acute testing. Due to the lack of toxic effects, US EPA waived the testing requirements in the areas of carcinogenicity, development, reproduction, immunotoxicity, and endocrine function. Due to the lack of any toxicity to freshwater organisms US EPA waived estuarine/marine organism testing.

In the acute freshwater fish testing, trout showed no effects at 1,000 ppm. The highest dose tested for freshwater aquatic invertebrates, in this case *Daphnia,* was >100 ppm.

Environmental Exposure

This pesticide risk assessment is complicated by the lack of data for this chemical. Being a low risk, practically non-toxic pesticide to vertebrates, US EPA waived some of the data requirements for registration. There are no physical parameters useful for calculating fate and transfer in the environment. It is estimated the half-life of the compound is 4 days in the field based on a half-life study. There is no current understanding of hydrolysis or other degradation processes for the compound. US EPA stated in its risk assessment that it anticipated the protein to lose its potency rapidly upon release in the environment because of its protein nature. In order to be effective, proteins must have a very specific conformation, or shape. This shape can be altered easily in solutions with different conditions, such as when dissolved in water versus venom fluid and this shape can also change when pH changes. The label of the product clearly states that the product must be mixed and applied within 24 hours, further adding to the understanding that this protein is not active very long after it's added to water.

In order to estimate the concentration that would be in the environment several assumptions were made. When data are not available a worst-case scenario is assumed. It was assumed that the compound does not degrade and that 100% of it moves off the targeted application site. The standard US EPA pond was used and the maximum application rate of the compound was applied to that pond. The US EPA pond is one hectare in size and 2 meters deep, containing 20,000,000 liters. The maximum rate on the currently registered product is “not to exceed 6 pints per year” with 2% active ingredient. Using these measurements, the calculated Expected Environmental Concentration (EEC) is 0.0028 ppm.

Conclusion

The EEC of 0.0028 ppm is much lower than the values produced by toxicity testing. The risk calculations suggest this pesticide is unlikely to cause nontarget effects in fish and other aquatic organisms; the EEC is over 35,000 times lower than the highest dose tested in aquatic invertebrates. Harmful non-target exposure would have to include very large quantities of the product before effects would be seen.

This is a newer insecticide and very few species have been tested. It is unlikely, but possible, that other non-target arthropods may have some toxicity to GS-omega/kappa-Hxtx-Hv1a. However, due to the short half-life and limited use it is expected that this product will breakdown rapidly in the environment and not reach concentrations that could pose risk to non-target organisms.

Isaria fumosorosea

Isaria fumosorosea is a fungus that occurs naturally throughout the environment worldwide. It can be found chiefly in terrestrial habitats; however, it can also be spread via water and air. The fungus is vectored by insects and mites. *Isaria fumosorosea* can infect many species including several agricultural pest insects like the diamondback moth and the Russian wheat aphid. Its use in Maine for browntail moth treatment is limited to fruit trees.

The risk assessment process for microbes is different than it is for conventional pesticides. The US EPA requires less fate and transfer information about pathogens like *I. fumosorosea* because of the worldwide distribution of this species. US EPA acknowledges that this pesticide will be able to cause harm to some insects however the concentrations used on the application site are expected to be low enough to be an acceptable risk to non-target insects nearby. *Isaria fumosorosea* pesticide formulations need to be kept at four degrees Celsius in order to be shelf stable; the product decays rapidly in sunlight and at warm temperatures.

Toxicity

A concern unique to microbial pesticides is the potential for the microbe to infect humans, birds, and other non-target organisms. In terms of its potential for infecting mammals, *I. fumosorosea* requires temperatures below 35 degrees Celsius to survive. The human body temperature is 37 degrees Celsius, therefore it is expected that *I. fumosorosea* cannot grow in mammals or birds because of their higher body temperatures. An additional concern evaluated by US EPA is the unintentional production of other components during the manufacturing process. *Isaria fumosorosea* manufacture was found to produce several other metabolites during processing, however, they were found to be less than 0.1% of the end product and US EPA does not consider them to pose undue risk.

Throughout toxicity testing, *I. fumosorosea* showed a low risk profile. *Isaria fumosorosea* is considered to be practically non-toxic to laboratory mammals in terms of ingestion, inhalation, injection into the abdominal cavity, contact with skin, and contact with the eye. However, it should be noted that dermal contact and contact with the eye can produce slight irritation that persists for a short period of time and is reversible. In terms of applicator safety this irritation potential is mitigated by the use of personal protective equipment such as long pants, long sleeves, gloves, and a respirator.

Two laboratory bird species were tested in an acute ingestion scenario and *I. fumosorosea* was considered to have acceptable risk to birds. Terrestrial insects that are not at the site of application are believed to have acceptable risk. *Isaria fumosorosea* showed toxicity to non-target terrestrial invertebrates at concentrations that were 10 to 1000 times higher than the Expected Environmental Concentration (EEC). Acute contact testing with bees demonstrated acceptable risk. Testing showed no effect at 10 times the EEC for both oral and contact exposures to bees. *Isaria fumosorosea* testing however was only performed in an acute setting and it is unknown about the longer-term pathogenicity to bees. US EPA expects the potential for pathogenicity to larval bees in chronic exposures to be mitigated by label directions specifying that this product should not be used on plants while they are in flower. The risk to terrestrial insects is mitigated by label language for reducing drift in non-target movement. *Isaria fumosorosea* was nonpathogenic to two species of marine invertebrates. There is no expectation of toxicity to fish.

Isaria fumosorosea has permanent exemption from tolerance for commodities in the United States.

Environmental Exposure

Isaria fumosorosea is not expected to persist in water for extended periods of time because soil, not water, is its habitat. As packaged, the pesticide can persist on a shelf for 12 months. The product decays rapidly in sunlight and room temperatures.

Conclusion

No toxicity is expected to occur in the marine and estuarine aquatic environment from labelled uses of *I. fumosorosea*. Rapid breakdown and low toxicity combine to keep the risks to non-target organisms acceptable.

Kaolin Clay

Kaolin clay is a pesticide commonly used in organic agriculture to manage a variety of pests including: mites, fungi, bacteria, and insects. Like many biochemical minimum risk pesticides kaolin clay has a nontoxic mode of action. Application of the product forms a layer that physically protects plant tissues from sunburn and pest destruction.

Kaolin clay is unique even among minimal risk pesticides because of its long history of use. Currently, kaolin clay is used in cosmetics, paperboard, adhesives, cellophane, toothpaste, antiperspirants, and anti-caking agents in food. These uses have given kaolin clay the GRAS (generally recognized as safe) categorization by the USDA.

Toxicity

Human exposure to kaolin clay stemming from its pesticidal uses is difficult to isolate. This product is found in thousands of consumer products making the ability to tease apart pesticidal influences and other influences impossible. These exposures however lead to the conclusion that this pesticide likely poses minimal risk to humans. Kaolin clay has been exempted by the US EPA for tolerance of residues when used on or in food commodities.

Despite the low likelihood of risk to applicators and bystanders, there is potential exposure to wildlife from the use of kaolin clay. Kaolin clay is a broad-spectrum pesticide which increases the potential for non-target effects. Typical use patterns, however, are what prevent significant non-target effects. This pesticide must be applied in a targeted fashion in order to be effective. Once it has shaken loose from its application site it returns to the soil simply as dust, and too dilute to affect organisms in the ecosystem. In honey bee testing studies, the toxic acute oral LD_{50} concentration of kaolin clay concentration was greater than 1,000 ppm and the acute contact LD_{50} concentration for honey bees was greater than 100 micrograms active ingredient per bee. These numbers indicate that kaolin clay is practically nontoxic to bees when used according to label directions.

In acute testing, kaolin clay is considered to have negligible toxicity to mammals. Toxicity testing of kaolin clay found the LD_{50} value in rats was greater than 5,000 ppm. Dermal and inhalation toxicity tests on mammals found similar results, all demonstrating low risk nature of kaolin clay. Kaolin clay can be irritating to the eyes in a temporary basis, but it is not corrosive to the eye. The US EPA considers the risk to eyes to be mitigated by the appropriate personal protective equipment. Because of the consistent lack of toxicity (including mortality and sublethal effects) in the acute tests, US EPA waived the chronic toxicity tests for covering developmental, reproductive, immunological, endocrine disruption, and carcinogenic effects. Testing with kaolin clay supports the lack of toxic effects to fish and aquatic invertebrates.

Environmental Exposure

There are virtually no data for modeling the environmental fate in transfer of kaolin clay because EPA waived those data requirements during its review. As a clay its half-life is indefinite and it is considered to be stable in the environment. Kaolin clay is known to be easily dispersed in water. In terms of chemical interactions, kaolin is chemically inert.

Conclusion

The toxic action of kaolin clay is not latent - its insecticidal effects are immediate and organisms must interact with it directly to be affected. It is a basic constituent of the earth and soil with many uses and has been used by

people to the degree it is now generally recognized as safe. Aquatic organisms in the Gulf of Maine are not at risk of undue harm from labeled uses of pesticides containing kaolin clay.

Spinosad

Spinosad is a widely used insecticide with a broad range of target pests and use sites. Commercial spinosad products are combinations of the spinosyn A and spinosyn D toxins. These toxins are produced by fermentation of the bacterium *Saccharopolyspora spinosa*. Spinetoram is another fermentation product of the same bacterium only differing from spinosad because it is the mixture of the spinosyn J and spinosyn L toxins. US EPA considers spinosad and spinetoram to be toxicologically similar for assessing human health endpoints but not for ecological endpoints. In the previous browntail moth risk assessment for pesticides used in the near marine shore zone, spinetoram was excluded from the 50- to 250-foot zone due to toxicity concerns for aquatic invertebrates.

Spinosad is a neurotoxin that has high selectivity for invertebrates. Its mode of action is to cause the excitation of neurons in the insect nervous system (via nAChR and GABA receptors) leading to excessive and uncontrollable neuron firing.

Toxicity

Spinosad can cause effects in most animals, however, the sensitivity to spinosad is highest in invertebrates (target and non-target) and aquatic organisms. Spinosad is considered to be moderately toxic to fish. Few marine or estuarine invertebrate species have been tested and for those that were tested there was a large range of sensitivity responses between species.

Acute: The only marine or estuarine fish to be tested were sheepshead minnows which displayed acute toxic effects at 7,870 ppb. For marine invertebrates, Eastern oysters were the most sensitive to acute exposures with an LC_{50} of 300 ppb. Among all aquatic invertebrates (fresh and marine/estuarine) the range in values seen in response to acute test exposures was 1.8 ppb to 51,700 ppb.

Chronic: The chronic effect threshold, called the No Observed Adverse Effect Concentration or NOAEC, for Sheepshead minnows was 1,150 ppb. In order to capture the range in variation of sensitivities for invertebrates, when the RQ was developed for chronic test exposures the arithmetic mean of all NOAEC values (fresh and marine/estuarine) was taken. Mysid shrimp displayed negative effects at 84.2 ppb in chronic tests. The 84.2 ppb for mysid shrimp is higher than most values and is an outlier when compared to the rest of the invertebrates; the lowest NOAEC was 0.5 ppb and the average without the 84.2 ppb value is 2.0 ppb.

Environmental Exposure

Spinosad has a relatively short persistence in the environment which largely depends on the amount of sunlight exposure it receives. Spinosad is stable in water but will degrade within hours in sunlit water. Similarly, spinosad can have half-lives of up to 47 days in soil degradation studies; however, field dissipation studies find the half-life is typically only a few days at most. In soil, spinosad is actively broken down by soil organisms.

A quantitative risk assessment approach was possible with spinosad. US EPA's Pesticide in Water Calculator was used to predict the Expected Environmental Concentration (EEC) under the standardized pond scenario frequently used by US EPA. The modeled peak concentration was 2.65 ppb based on the predicted drift and runoff from an application to a modelled apple orchard with air blast sprayers. The calculated RQ for an acute exposure was 0.009; any RQ value higher than 0.05 is classified as unacceptable risk for listed species. The modeled 21-day average EEC was 2.12 ppb. The value for Eastern oysters and the average value of all chronic test exposures were used for the chronic RQ calculation, 300 ppb and 12.29 ppb respectively. The calculated RQ for chronic exposures was 0.17; in this case any RQ value higher than 1 is considered unacceptable risk. This

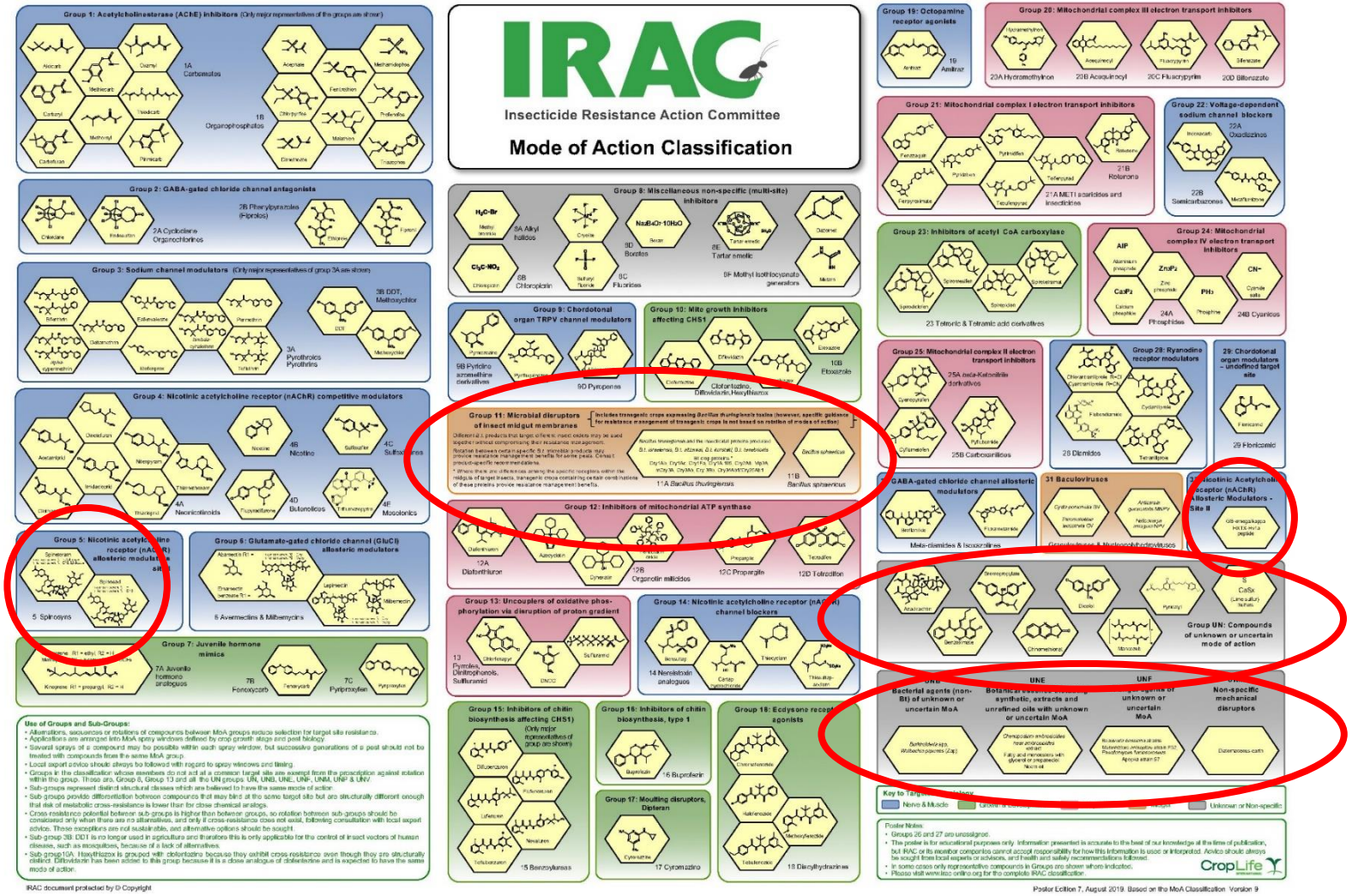
assessment used the strictest criteria for assessing risk by assessing the RQ values at the level used endangered organisms.

Conclusion

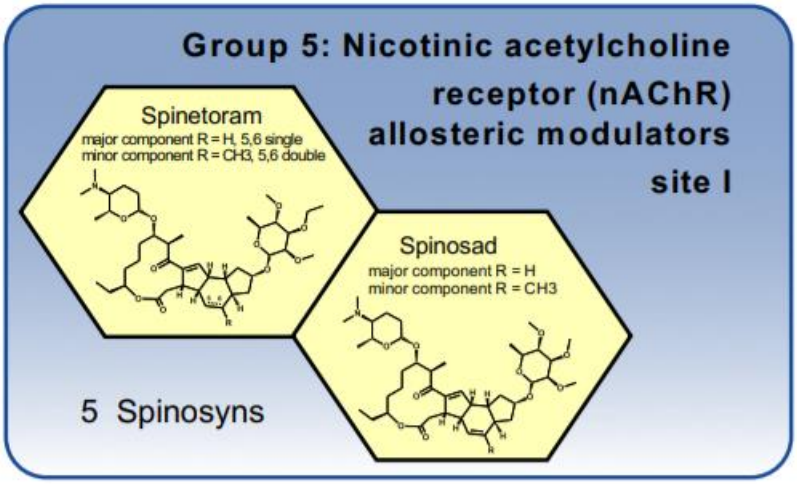
Spinosad is not expected to cause undue harm to the environment, and specifically to marine or estuarine organisms, when used as labeled. Spinosad can produce toxic effects, as seen when tested on aquatic fish and invertebrates. However, these effects are only seen at high concentrations and the labelled uses are currently protective.

Resistance Management Information

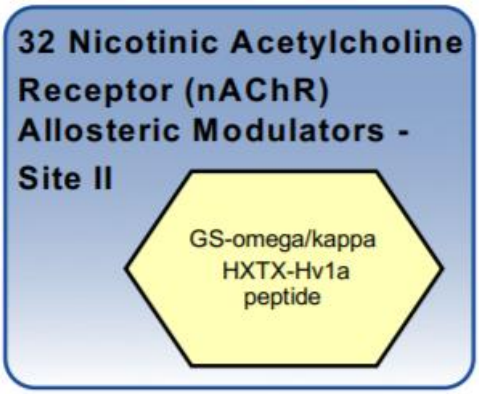
Seven unique IRAC groups are associated with this group of possible “biological pesticides”. Each circled area is shown larger on the pages that follow.



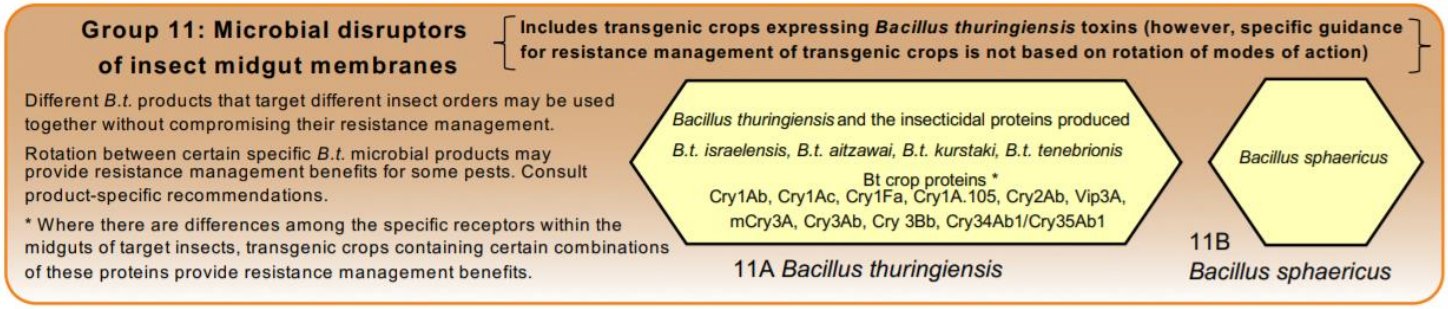
Spinosad is in IRAC Group 5.



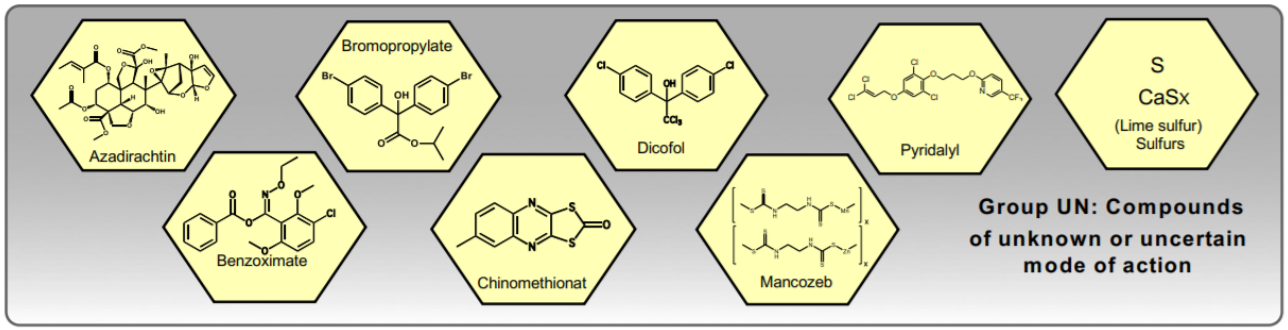
GS-omega/kappa HXTX-Hv1a is in the new IRAC Group 32.



***Bacillus thuringiensis* is in IRAC Group 11.**

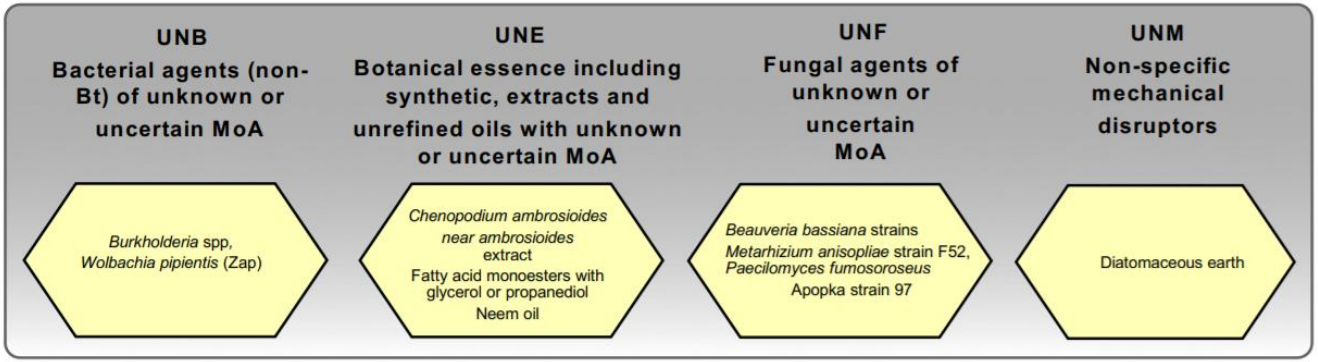


Azadirachtin is in IRAC Group UN.



***Beauveria bassiana* & *Isaria fumosorosea* are in IRAC Group UNF.**

Kaolin clay is in IRAC Group UNM.



Updated Policy

An updated draft mock-up of the policy for allowable “biological pesticides” for the purposes of Chapter 29 Section 5, follows on the next page. This definition of “biological pesticides” is limited to application of pesticides on the length of land between the 25 to 50 foot marks from the high-tide line. In summary,

> This risk assessment document provides a list of biological pesticides for consideration to be used in the zone of land 25 to 50 feet from the high-tide line during the treatments for browntail moths, potential active ingredients include:
>
> > *Bacillus thuringiensis kurstaki* & *aizawai*
> > *Beauveria bassiana*
> > GS-omega/kappa-Hxtx-Hv1a
> > *Isaria fumosorosea*
> > Kaolin Clay
> > Spinosad
>
> This risk assessment document supports the removal of azadirachtin from the group of active ingredients currently allowed under the “biological pesticides” grouping.

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BOARD OF PESTICIDES CONTROL
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COMMISSIONER

MAINE BOARD OF PESTICIDES CONTROL POLICY—DEFINITION OF BIOLOGICAL PESTICIDE AS IT RELATES TO CHAPTER 29 SECTION 5

Revised Month Day, 2020

BACKGROUND

The Board discussed questions that arose during the spring of 2016 relative to interpretation of the term "biological pesticide" as used in Section 5 of Chapter 29, which regulates pesticide applications for control of browntail moth adjacent to marine waters. The staff pointed out that when this rule was originally written, it contemplated that "biological pesticide" would primarily include strains of *Bacillus thuringiensis* and similar microbial pesticides. With the recent increase in browntail moth populations, questions have arisen about other active ingredients which are derived from organisms. Staff indicated that the term "biological pesticide" is now commonly perceived to include pesticide active ingredients consisting of single cell organisms or products derived from organisms. At the January 11, 2017 meeting, the Board reviewed various options and adopted an interpretation of the term "biological pesticide," which was subsequently amended at the March 31, 2017 meeting.

In 2019, continued interest in expanding the number of available biological pesticides prompted the BPC to re-evaluate the list of biological pesticides. Qualitative and quantitative risk assessments were used to determine the active ingredients appropriate for this use.

POLICY

For the purposes of Chapter 29, Section 5, the term "biological pesticide" includes any microbial pesticide that contains the microorganism and byproducts normally associated with the organism, as approved by the Board.

As of Month Day, 2020 the Board has approved:

Bacillus thuringiensis subspecies *kurstaki*
Bacillus thuringiensis subspecies *aizawai*
Beauveria bassiana
GS-omega/kappa-Hxtx-Hv1a
Isaria fumosorosea
Kaolin Clay
Spinosad

Grey out text TO BE DETERMINED

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