
Light Brown Apple Moth (LBAM) Eradication Program: Potential Effects on Pollinators and Implications for California Agriculture

Summary Report Prepared by

Roy Upton LBAM Liaison Citizens For Health Soquel, CA	Daniel Harder PhD Department of Evolutionary Biology University of California Santa Cruz, CA	Thomas Dadant Santa Cruz, CA
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Introduction

Worldwide there has been an alarming incidence of *Colony Collapse Syndrome* among honey bee populations. Some states within the United States have lost up to 90% of their bees and the trend does not seem to be reversing. According to the United States Department of Agriculture (USDA), one-third of the human diet is derived from insect-pollinated plants and that honeybees are responsible for 80 to 85% of this pollination. Pesticides can severely impact bee colonies and are considered to be one of the four primary stressors on bee colonies. Microencapsulated pesticide delivery systems, such as are used in the LBAM eradication program, are considered to present a more specific and detrimental action on bee colonies than standard spray applications due to the size of the microcapsules being within the exact same size range (10 to 190 microns) as pollen grains (10 to 115 micron). In addition to the potential of the wings and bodies of bees getting soaked in the physical sticky viscous liquid associated with these microencapsulated pesticides and therefore having their flight inhibited, microcapsules can electrostatically stick to the pollen gathering hairs on the bees. The bees gather and carry pesticide-laden microcapsules to the hive where the contents are fed to the larva, brood, emerging adults, and the queen. The pesticides encased in the microcapsules used in the LBAM spray, which include compounds that are directly toxic to bees, are designed to break down over a period of from 30 to 90 days thereby presenting a constant exposure of pesticides to the hive throughout 2 to 3 lifecycles of the bees (25 to 45 days).

Regarding the light brown apple moth (LBAM) eradication program, and the widespread use of pesticides across agriculture and residential areas there has been a significant amount of attention made of the potential for human adverse health effects and very little paid to the potential environmental effects, including the effects of such broad based aerial pesticide spraying on bees specifically and pollinators. A previous LBAM Fact Sheet addressed the temporal association and toxic mechanistic plausibility between the aerial spray and the death of more than 650 seabirds, all of which died within days of the aerial spraying in Santa Cruz and starting the day immediately after the first spray. After the aerial spray of the Checkmate pesticide solution in Santa Cruz, CA, an anomalous and temporal association of disoriented and dead bees was observed by many people but went uninvestigated by State and Federal officials.

A large percentage of agriculture products in California depend on pollinators, most specifically honey bees. Bee pollination in California itself is a \$10 to \$14 billion business and has residual economic impact on numerous crops as well as California's \$14.5 billion dairy industry. Even a minor negative effect of the LBAM eradication program, which in addition to the aerial spraying of pesticide solutions includes chlorpyrifos, *Bacillus thuringiensis* (Bt), permethrin, and spinosad, all of which are highly toxic to bees, can have devastating negative effects on California agriculture that is far greater than any real or projected estimates from damage due to LBAM, which is considered very low. In light of the severe incidence of *Colony Collapse Syndrome* throughout the United States and important economic impact of bees on California agriculture, it is imperative that State agriculture officials reconsider the use of microencapsulated delivery systems for pesticides and call a halt to the LBAM eradication program before there is greater damage to California bee populations.

Methodology and Contacts

The opinions in this report were based on the primary scientific literature regarding bees and their value to agriculture as well as the threat they face from pesticides in general and microencapsulated delivery systems specifically. Specific observations of the detrimental effect of the Checkmate pesticide spray solution in Santa Cruz are discussed. We also address the basic toxicology of the ingredients in the Checkmate pesticide solution, specifically as they relate to toxicity to bees as well as the potential for toxicity of other pesticides used in the LBAM eradication program, which include; chlorpyrifos, *Bacillus thuringiensis*, and spinosad.

Importance of Bees in California Agriculture and Dairy

A 2000 Cornell University study concluded that the direct value of honey bee pollination to U.S. agriculture is more than \$14.6 billion, while other sources put it at \$24 billion. California has the largest beekeeping industry of any state in the United States. Nearly 500,000 colonies of bees are operated by 400 commercial and semi-commercial beekeepers. Most beehives are rented one or more times annually for pollination of agricultural crops. Nearly 75% of the country's documented commercial honey bee crop pollination occurs in California and California is a leader in the production of honey and beeswax, with revenues of \$52.3 million just in these two products alone and direct beekeeping income exceeding \$126 million in 2002 (Mussen 2004). When all direct and indirect revenues are calculated, honey bees are responsible for nearly half of California's agricultural production (cash receipts for farm marketing), which is currently valued at more than \$30 billion (Mussen 2004).

Honey bees are needed for the pollination of many fruit and vegetable crops, as well as for native plants that maintain a healthy ecosystem rich with biological predators against pests, such as LBAM. Commodities requiring pollination or cross-pollination between varieties to produce crops include almonds, apples, sweet cherries, plums, and prunes, and pollination is required for other crops as diverse as avocados, blueberries, cherries, cucumbers, melons, pumpkins, and squash to name only a few. According to the USDA, one-third of the human diet is derived from insect-pollinated plants and honey bees are responsible for 80-85% of this pollination (Sanford 2003).

In California specifically, at least 21 fruit and nut crops produce larger yields when pollinated by honey bees. These fruit, nut, and vegetable crops were worth \$4.4 billion in 2002. Bees are specifically critical to California almond crops, which yield in excess of \$2.5 billion annually and have become the nation's most valuable horticulture crop, double the sales of California wines. Pollination of almond trees requires the importation of more than half of the number of honey bees that are in the US (Agnew 2007). Bees are also critical to alfalfa and hay, which support a \$4.5 billion dairy industry (HR 110). Bee pollination itself is a \$10 to \$14 billion business. Bees produce honey and beeswax, bringing in \$285 million dollars annually. Additional bee products such as pollen, propolis, royal jelly, and bee venom, all contribute significantly to California's economy (GLW 2007).

Colony Collapse Syndrome

The importance of bees to the world's agriculture supply and to the California economy cannot be overstated. Taking active steps not to decimate bee populations is equally critical in the face of emerging *Colony Collapse Disorder*, which has resulted in a loss of more than 1/3 of honeybee colonies in 2005. Some states have lost more than 90% of their bee colonies (GLW 2007). Pesticides can severely impact bee colonies and are considered to be one of the four primary stressors on bee colonies. Yet, the EPA only requires that pesticides be assessed for adult bees, neglecting any effect pesticides may have on the brood and immature bees (COA 2007). In the case of the pheromone pesticide solutions approved for use in the LBAM eradication program, no

tests on bees were performed. Moreover, individual pesticides may not be found to be injurious to bees but when bees are exposed sequentially to an array of pesticides that may be in the environment, these collective pesticides may become lethal (COA 2007).

A similar phenomenon has been reported throughout Europe, the Mediterranean, Spain, and Taiwan. While a single definitive cause of *Colony Collapse Disorder* has not been identified, and likely will not be, there are numerous contributing factors that have been associated with it and these include changes in the environment, malnutrition, disease (e.g. Israel acute paralysis virus), mites, pesticides, and foods genetically modified with pest control characteristics (e.g. transgenic corn). Aside from being a potential causative or contributor to *Colony Collapse Disorder* pesticides in general are considered to be among the top four stressors to bee hives, which are particularly sensitive to even small amounts of toxic chemicals. Microencapsulated delivery systems present an especially toxic threat to hives as the size of the microcaps, which are filled with the pesticide contents, are within the exact same size range as pollen grains (15 to 100 microns). While the USDA and State have alleged the concentrations of the potentially toxic inert ingredients are too low to negatively effect humans and marine life, they failed to perform any studies that would suggest the concentration of inerts being slowly released over time and directly fed to bees would not harm the bees or other pollinators.

Microencapsulated Delivery Systems

By far the most potentially damaging pesticides affecting honey bees thus far developed are those packaged in microcapsules, which present a very distinct and serious threat to bees (Sanford 2003; Taryp 2008). Entire hives have been killed due to such delivery systems (Adams 2008). There are a number of reasons why microcapsules are a specific threat to bee colonies. First is that the microcapsules solution used in the aerial pesticide application of Santa Cruz was sticky and viscous, probably due to the combination of the physical capsules mixed with emulsifiers (e.g. polyvinyl alcohol) and surfactants (e.g. tricapyryl methyl ammonium chloride). This material was found to stick on plants, cars, spider webs, windows, planter boxes, kayaks, and house decks. Microcapsules are also designed to remain in a relative state of suspension creating ambient saturation of pheromone solution, otherwise the solution will not work for mating disruption. This sticky solution can coat bees as they gather pollen and nectar. In Santa Cruz, people reported seeing bees flying in a disoriented fashion, struggling on the ground, and many dead the morning after the spray. Conceivably this could have been due to the physical stickiness and weight of the solution impeding their ability to fly. Secondly, as noted, microcapsules are the exact same range of size as pollen grains (15 to 100 microns). The microcapsule size in the Checkmate delivery system is 10 to 190 microns (Werner et al. 2007; see Figure 1) and according to CDFA, a percentage of the microcapsules are even smaller than 10 micron in diameter (Leavitt 2008). These were applied at a rate of approximately 135 microcapsules per square foot (maximum of 809 microcapsules per foot) (CDFA 2008). Because of their size and shape similarity with pollen, microcapsules with their pesticide contents are carried back to the hive where it is combined with pollen that is being stored as food. Microcapsules are inherently designed to release their chemical contents slowly over a period of days or weeks. The microcapsules of the Checkmate solution used in the Monterey and Santa Cruz pesticide sprays are designed to break down in 30 to 90 days. The pesticide contents are then fed to the queen, brood, and emerging adults (Ferrel and Aagard 2005; Kelly et al. 2002; NPARU 2008) potentially leading to the devastation of every hive that was exposed. Even at small concentrations the negative effects of pesticides to hives can be significant. Additionally, the toxicity of pesticides can be greatly increased through microencapsulated delivery systems, likely due to the presence of surfactants and emulsifiers, which can enhance absorbability. For example, the organophosphate pesticide methyl parathion is extremely toxic to bees with normal application. But, when it began to be marketed in a microencapsulated delivery system (PennCap-M) the inherent toxicity was greatly enhanced and was reported to at least partly result in "very severe poisoning" among hives in Washington state (Deryckx 1977; Wilson et al. 2002).

Among bees, the workers are those primarily affected by pesticides. The symptoms of poisoning can vary depending on the developmental stage of the individual bee and chemical to which they are exposed (see Table 1) (Sanford 2003). From an environmental perspective, microencapsulated pesticides should never be used if there is any chance bees might collect the microcapsules (Sanford 2003), a premise that is impossible to guarantee with aerial pesticide spraying.

Figure 1 Example of microcapsule delivery system (left) and pollen grains (right)

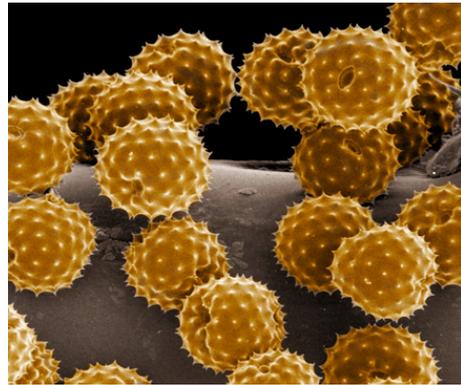


Table 1 Developmental Stage of Bee Maturity

Development of adult: It takes worker bees about twenty-one days to develop from egg to adult. During this process, each individual passes through a larval (feeding) stage followed by a pupal (transformation) stage. The larval stage is the most susceptible to pesticide poisoning during development.

House bees: These bees are emerged worker adults up to twenty-one days of age. They care for the brood, process pollen and nectar gathered in the field by older workers, and clean the nest. Eventually, they too will become field bees. House bees are usually poisoned by contaminated pollen, which is collected in the field, brought back and stored in the hive. As house bees are killed, there are fewer bees to tend the brood and further decline in population results.

Field bees: These bees are workers twenty-one to approximately forty-two days of age. There appears to be no greater risk in bee society than to be a field bee. Should the insect avoid all the potential pitfalls due to predators like spiders, toads or skunks, it is still vulnerable at all times to the numerous pesticides applied in commercial agriculture, mosquito control, and home gardens. Most times, field bees are killed by contact with pesticides in the field, but other times they collect contaminated nectar and pollen and contribute to poisoning their sisters in the colony. If field bees are killed, then young bees are forced into the field earlier than normal, disrupting and thus disorienting the colony.

Review of Toxicity of Checkmate Aerial Pesticide Solution to Bees

The Checkmate LBAM-F is a cocktail of chemical toxins, 2 pheromones, surfactant (tricaprylyl methyl ammonium chloride; aka Aliquat 336), plastic resins, an antioxidant, and emulsifier. The solution is designed to hang in the air to maintain an ambient saturation of pheromone and to stick to surfaces, lest it all fall to the ground, which would render it ineffective as a pheromone disruptor. The moths mate higher in the air not at ground level. This sticky material can simply adhere to the wings and bodies of bees and butterflies making it difficult or impossible to fly, as apparently observed in Santa Cruz. Microencapsulated pesticide solutions have the potential to negatively affect thousands of different insects with untold ecological consequences.

When testing pesticide solutions, only the active ingredients are often tested. However, research suggests that the so-called “inert” ingredients in a pesticide are often among the most toxic. The word “inert” as used on a pesticide label is commonly mistaken to mean inactive or benign. However the EPA states that “although the term “inert” may connote physical, chemical or biological inactivity, use of the word “inert” to describe a component in a pesticide product means only that the substance is not intended to exert a pesticidal effect in that product. The “inert” ingredient may have biological activity of its own, “it may be toxic to humans, and it may be chemically active” (EPA 2002). Typically, pesticide formulations are comprised largely of inert ingredients. A review of 100 agricultural pesticide products found that the formulations contained on average 50% inert ingredients, with many containing 90% or more (NCAP 2006). The majority of safety tests required to register a pesticide are performed with the active ingredient alone, not the complete formulation (Cox and Sorgan 2006). The Checkmate LBAM-F formulation consists of 17.61% moth hormonal disruptors and 82.39% other ingredients (see Table 2).

Numerous studies have shown that inerts can increase the toxicity of pesticides to body systems such as the nervous, cardiovascular, and hormonal systems, the mitochondria, and genetic material. Inerts can also interact with other chemicals in pesticide formulations, to increase human exposure levels to the active pesticide. Additionally, inerts have been shown to raise the ecotoxicity of pesticide formulations, increasing the severity of toxic effects to plants, animals, and non-target microorganisms (Cox and Sorgan 2006), such as bees.

State and Federal Agencies have alleged that the Checkmate LBAM-F formula, consisting of pheromones as active ingredients, is an environmentally safe product with no known negative human or environmental effects because pheromones are abundantly available in the environment. It is correct that pheromone based pesticides are more environmentally sound than organophosphate pesticides and that natural insect pheromones are abundant in nature. However, the pheromone being used is a synthetic product that mimics the effects of the natural pheromone so while the synthetic may be considered similar to the naturally produced pheromones it cannot be considered identical as moths relatively quickly learn to distinguish true from synthetic pheromone. The greatest concern regarding the safety of the pheromone portion of the formulation is that it has not been tested with regards to what effects it may have on non-target organisms. CDFA has noted that at least 5 moths other than LBAM are being found in the LBAM traps suggesting a potential effect on non-target species, to what degree is unknown. There is significant concern regarding the so-called “inert” ingredients in the Checkmate formula, most of which have a detrimental effect on bees. A review of the available data for these chemicals indicates a high potential for toxicity for many of the ingredients labeled as inert, even at low concentrations. Moreover, the degradation products of a number of the Checkmate inert ingredients are more toxic than the parent compound. In addition, all of the other treatments being used in the LBAM eradication program are highly toxic to bees. Because, CDFA declared this program an emergency, environmental impact reviews were not conducted so the environmental consequences of the combination of pesticide products being used is completely unknown.

The following toxicological information was derived from database reviews, primary published scientific literature, and Material Safety Data Sheets (MSDS). A MSDS is designed to provide workers and emergency personnel with the proper procedures for handling or working with potentially toxic substances. MSDSs include information such as physical data (melting point, boiling point, flash point etc.), toxicity, health effects, first aid, reactivity, storage, disposal, protective equipment, and information regarding environmental accidents such as spills or accidents. The information presented provides a partial overview of the toxicity of the compound with any known effect specifically reported about ecological toxicities and toxicities to bees.

Table 2 Ingredients in Checkmate LBAM-F and OLR-F

Water	
(E)-11-Tetradecen-1-yl acetate	16.9% (pheromone)
(E,E)-9,11-Tetradecadien-1-yl acetate	0.71% (pheromone)
(z)-11-Tetradecenyl acetate (pheromone)*	
11-Tetradecen-1-ol acetate (pheromone)	
Tricaprylyl methyl ammonium chloride (syn. methyltrioctylammonium chloride)	
Sodium phosphate	
Ammonium phosphate	
1,2-benzisothiazoli-3-one	
2-hydroxy-4-n-octyloxybenzophenone	
Butylated hydroxytoluene	
Polyvinyl alcohol	
Cross linked polyurea polymer	
Polymethylene polyphenyl isocyanate*	

* The actual presence of this compound in the solution has been questioned. It may be used as a building block for the crosslinked polyurea polymer. (z)-11-Tetradecenyl acetate is a component of Checkmate OLR-F.

a. Tricaprylyl Methyl Ammonium Chloride (synonym methyltrioctylammonium chloride): CAS Number: 5137-55-3 (TMAC)

Also known by the trade name Aliquat 336 (Acros MSDS; de Oliveira and Bertazzoli 2007; Sigma-Aldrich MSDS) tricapyrylyl methyl ammonium chloride (TMAC) is a low-foaming surfactant that keeps polymer beads from sticking together. Surfactants in general allow other compounds to dissolve in water and change the surface tension of water (Abraham 2007; Gyenge and Oloman 2001; de Oliveira and Bertazzoli 2007). This effect on water can affect zooplankton and even at low doses can significantly impact amphibians such as frogs (Abraham 2007). European labeling warns against releasing the substance into the environment, cautioning that it may cause long-term adverse effects in the aquatic environment. Surfactants can increase the systemic toxicity of substances through increased absorption.

b. 1,2-Benzisothiazoli-3-one (synonym BIT); CAS Number: 2634-33-5

1,2-Benzisothiazolin-3-one is a preservative associated with occupational asthma. Multiple accounts of occupational dermatitis have been reported with exposure to the chemical. In the European Union, it is classified as irritating to the skin and as a potential risk of causing serious eye damage. Canadian authorities list it as causing skin sensitization in humans. BIT is a known dermal irritant at levels as low as 0.1% concentration and individuals with dermal conditions should avoid repeated exposure to BIT (Damstra et al. 1992; Muhn and Sasseville 2003; Roberts et al. 1981; Taran and Delaney 1997). Individuals with chronic pulmonary or asthmatic conditions or chronic skin conditions are warned to avoid repetitive exposure to BIT. Symptoms of exposure include respiratory tract and mucous membrane irritation, severe eye irritation, skin irritation, and dermatitis. According to data compiled by the Occupational Safety and Health Administration (OSHA) BIT has been shown to be a mutagen with genotoxicity to human cells.

In the European Union BIT is classified as dangerous to the environment and as very toxic to aquatic organisms with specific negative effects against mollusks, fish, and zooplankton. It is highly toxic to green algae and can disturb aquatic ecosystems. According to the EPA, it has a low to moderate toxicity to birds and mammals, a moderate toxicity to fresh water fish and invertebrates (starfish, crabs, insects), and is highly toxic to estuarine and marine habitats. European labeling warns against releasing the substance into the environment. It is classified as "hazardous waste" by the European Waste Catalogue Ordinance and as a "hazard to waters" by the European Administrative Regulation of Substances Hazardous to Water. Domestic MSDS sheets for BIT warn that water polluted with the substance should not be discharged into sewage or natural areas. Documents of the EPA on BIT state that it is highly toxic to green algae and other invertebrate species. The EPA also states that if it is used outdoors, BIT may possibly move with soil during rainfall events and potentially reach surface waters. The Santa Cruz county sprayings on November 8th and 9th were followed by a significant rainfall on November 10th and

11th. The rainfall was associated with an anomalous yellow runoff from the land into the Monterey Bay via several drainpipes. This runoff was yellow and sticky and left a thick layer of foam on top of the water for miles along the Santa Cruz shore. No testing of this runoff was performed by State or Federal Agencies.

c. 2-Hydroxy-4-n-octyloxybenzophenone (synonym benzophenone 12); CAS Number: 1843-05-6

2-Hydroxy-4-n-octyl benzophenone is a UV light absorber of unknown health impact, however related compounds in the benzophenone family have been shown to form estrogenic photoproducts, upon exposure to UV or sunlight (Hayashi et al. 2006). In the European Union it is classified as an irritant that may cause sensitization upon skin contact and is irritating to the eyes, respiratory system, and skin. Symptoms of exposure include reddening and irritation of the skin and eyes, mucous membrane irritation, and upper respiratory tract irritation.

2-Hydroxy-4-n-octyl benzophenone is classified as harmful to aquatic organisms and may cause long-term adverse effects in the aquatic environment. European labeling warns against releasing the substance into the environment. It is classified as hazardous by OSHA.

d. Butylated Hydroxytoluene (BHT) (synonym 2,6-Di-tert-butyl-p-cresol): CAS Number: 128-37-0

Butylated hydroxytoluene (BHT) is classified as irritating to the eyes, respiratory system, and skin in the European Union. Allergic contact dermatitis and contact urticaria are associated with exposure to BHT (HAZ-MAP). Studies have shown BHT to be carcinogenic, hepatotoxic, tumorigenic, mutagenic, and teratogenic in animals as well as in human cells (Sigma-Aldrich MSDS). Studies have also confirmed BHT to have estrogenic activity (Miller et al. 2001; Wada et al. 2004) and MSDS sheets state that chronic exposure to BHT may cause adverse reproductive and birth defects (Acros MSDS). BHT is classified by OSHA as an ecological toxin with specific toxicity to marine life. It is a known eye and skin irritant and can cause a multitude of respiratory symptoms (e.g. cough, sore throat).

e. Sodium Phosphate

Sodium Phosphate (SP) (Disodium Phosphate): CAS Number: 7558-79-4

Sodium Acid Phosphate (SAP) (Monosodium Phosphate): CAS Number: 7558-80-7

Trisodium Phosphate (TSP) (Sodium Phosphate): CAS Number: 7601-54-9

There are a number of different forms of sodium phosphate. The exact type of sodium phosphate used in the Checkmate formulas has not been publicly disclosed, and therefore it is not possible to give a precise description of potential adverse effects. However, a review of the most common forms of sodium phosphate share similar toxicity profiles and it would be expected that the range of exposure symptoms would vary from mild to severe depending on the specific type of sodium phosphate used in the formula. Symptoms of exposure to the various kinds of sodium phosphate would range from mild to severe gastrointestinal effects (varying degrees of gastrointestinal irritation, abdominal pain/cramping, vomiting, diarrhea, nausea, abdominal discomfort, burning sensation), mild to severe respiratory symptoms (throat irritation, respiratory tract/mucous membrane irritation, coughing, sneezing, choking, difficulty breathing, pulmonary edema), mild to severe effects on the eye (irritation, redness, pain, conjunctival edema and corneal clouding followed by subsequent cataract formation could occur) eye burns, and mild to severe skin symptoms (skin/mucous membrane irritation, dermatitis, local skin destruction, burning pain, skin burns, blisters).

Environmentally, these compounds are classified as hazardous substances with potential detrimental effects on ground water and aquatic ecosystems. These most especially affect blue gill sunfish, rainbow trout, crustaceans, mollusks, and phytoplankton and zooplankton that can contribute to red tides (Feyzioglu and Ogut 2006), which in turn are toxic to marine habitats.

Sodium Phosphate (SP): Classified as a hazardous substance on California Director's List of Hazardous Substances & CERCLA (Science Lab MSDS). May cause irritation of the digestive tract and may cause purging. It is slowly absorbed. Expected to be a low ingestion hazard for usual industrial handling. Ingestion of large doses may affect behavior/central nervous system. If

a significant amount of phosphate is absorbed, hypophosphatemia will occur (Science Lab MSDS). SP is extremely caustic to eyes.

Sodium Acid Phosphate (SAP): Considered a low hazard for usual industrial handling and systemic reactions are unlikely when ingested (because they are slowly and incompletely absorbed in the intestinal tract). The most frequently seen effect is gastrointestinal irritation with abdominal pain and cramping, vomiting, diarrhea. If a significant amount of phosphate is absorbed. The following symptoms may occur: mineral imbalance in the body, adversely affecting the osmotic pressure of body fluids resulting in hyperphosphatemia, hypocalcemia, hypomagnesemia (Science Lab MSDS).

Trisodium Phosphate (TSP): Classified as "hazardous waste" under the European Waste Catalogue Ordinance (AVV) (Gestis Database); classified as a hazardous substance on California Director's List of Hazardous Substances, CERCLA, and OSHA (Science Lab MSDS). May be harmful if swallowed and may cause severe gastrointestinal (digestive) tract irritation with severe nausea, vomiting, abdominal discomfort, violent purging, diarrhea, and burning sensation. Ingestion of large amounts may induce hypocalcemia or hyponatremia characterized by tetanus-like spasms, due to the sequestration of calcium ions by the phosphate moiety. It may also cause caustic burns of the mouth oropharynx, esophagus, or gastrointestinal tract. TSP is extremely caustic to the eyes.

In general, sodium phosphate is a pH buffer. If runoff concentrations are high enough sodium phosphate could contribute to a change in water pH and lead to algal blooms (Abraham 2007) that can give rise to red tide. Increased phosphate levels are known to be a contributing factor in the occurrence of red tides (Feyzioglu and Ogut 2006). It may also be hazardous to drinking water when large quantities get into groundwater.

Following the sprayings in Monterey and Santa Cruz counties, a large number of the reported human adverse effects reported were consistent with the adverse effects profile of these various compounds. Similarly, a harmful algal bloom (red tide) described by a water specialist with the Santa Cruz County Environmental Health Services as "one of the more dramatic ones in recent memory", occurred in the Monterey Bay (Ragan 2007) four days after the spray. More than 650 dead seabirds were found from the day immediately following the spray to the several days following the spray including the days associated with this dramatic red tide. The temporal association and mechanistic plausibility between the actual spray and the dead and injured sea birds suggests more than a coincidental occurrence.

f. Ammonium Phosphate

***Monoammonium Phosphate*: CAS Number: 7722-76-1**

***Diammonium Phosphate*: CAS Number: 7783-28-0**

The exact type of ammonium phosphate used in the Checkmate formulas is currently unspecified, and could be either *monoammonium phosphate* or *diammonium phosphate*.

Monoammonium phosphate can cause mild respiratory tract irritation, nausea, vomiting (after inhalation of high concentrations of dust), coughing, shortness of breath, mild irritation, redness, and pain of eyes. Classified as hazardous by OSHA (Science Lab MSDS).

Diammonium phosphate is toxic to lungs and mucous membranes and can cause irritation to the respiratory tract, coughing, shortness of breath and eye inflammation characterized by redness, watering, itching, and pain. Characterized in Canada as very toxic. Repeated or prolonged exposure can produce target organ damage and cause damage to lungs and mucous membranes. Classified as hazardous by OSHA; long term degradation products may arise and products of degradation are more toxic than the parent compounds (Science Lab MSDS). May be a hazardous to drinking water when larger quantities get into groundwater (Gestis Database).

Following the sprayings in Monterey and Santa Cruz counties there were numerous reports of respiratory symptoms including asthma, bronchial irritation, difficulty breathing, shortness of breath, coughing and wheezing, lung congestion/soreness, and chest pain/tightness. Nausea, blurred vision, eye irritation, and skin rashes were also reported (HOPE 2008).

g. Polyvinyl Alcohol (PVA)

CAS Number: 9002-89-5

Polyvinyl Alcohol (PVA) is an emulsifier that allows other compounds to mix together and may keep the microcapsules suspended in water. The Society of Plastics Industry considers it a plastic resin. There is limited human data regarding the toxicity of polyvinyl alcohol. Animal data has shown it to be tumorigenic (Science Lab MSDS). Inhalation or ingestion of PVA for a prolonged period of time may affect blood, metabolism, and behavior (Science Lab MSDS). Symptoms of PVA exposure include digestive tract irritation, respiratory irritation or cough, and red/irritated eyes.

According to the National Institute of Occupational Safety and Health (NIOSH) polyvinyl alcohol may be hazardous in the environment, with special attention given to fish. It may also be hazardous to ground water (Gestis Database). It is considered to be harmless in isolation, but PVA could potentially dissolve other compounds on impervious surfaces into runoff.

Following the sprayings in Monterey and Santa Cruz counties there were numerous adverse effects reported, including nausea, diarrhea, coughing, wheezing, and eye irritation (HOPE 2008) as well as an anomalous runoff of yellow sticky substance that was observed coming from runoff drain pipes, in back yards, the rivers, and which accumulated in the Monterey Bay in the form of a thick yellow foam floating on top of the water along West Cliff Beach. Dead and injured birds were found with this sticky substance. It is possible this thick yellow sticky substance was an accumulation of the billions of microcapsules that were dispensed, mixed with the surfactants and emulsifiers that can dissolve other compounds on impervious surfaces (oils, other chemicals, pollutants) during the rainfall and keep them in suspension in the water, which is a function of emulsifiers.

h. Crosslinked Polyurea Polymer and Polymethylene Polyphony Isocyanate (PPI)*

CAS Number: information not available

According to Checkmate manufacturer Suterra, polymethylene polyphenyl isocyanate is used to create the encapsulation polymer that makes up the shell of the microcapsule that contains the Checkmate solution. The PPI starter compound is reported by the manufacturer to be used up during the reaction (Renner 2007). The *Consensus Statement* states that the polyurea shell biodegrades into urea. Research has linked urea to the occurrence of harmful algal blooms (HAB's), also known as red tides. Following the spraying, a harmful algal bloom (red tide) described by a water specialist with the Santa Cruz County Environmental Health Services as "one of the more dramatic ones in recent memory", occurred in the Monterey Bay (Ragan 2007).

Summary

The negative impact of pesticides on bee colonies is firmly established, as is the uniquely detrimental impact of microcapsule delivery systems. The inert ingredients that are present in the microcapsules, most especially tricapyryl methyl ammonium chloride are potentially toxic to bees who will be fed these compounds for long periods of time. If the impact of these pesticide treatments were to even have a marginal negative effect on the vitality of California bee colonies, the associated costs would dwarf any damage that could be realistically expected from LBAM and have negative effects on California wild flora for decades.

Review of Toxicity to Bees of Other Pesticides Used in the LBAM Eradication Program

In addition to the basic toxicity of the inert ingredients in the sprays that were applied and are projected to be applied (e.g. Checkmate LBAM-F), there are tremendous potential environmental consequences in the other products that are being used or, are projected to be used, as part of the LBAM eradication program. Most of these other products are directly insecticidal and directly toxic pesticides, most of which are known to be extremely toxic to bees in general and pollinators in general. Moreover, a relatively recent study from Canada reported that low level exposure of

pesticides to bees while in the larval development stage has an adverse effect on the adults that are not detected with current tests required by regulatory agencies (Morandin et al. 2005).

a. *Bacillus thuringiensis* (Bt)

Bacillus thuringiensis (Bt) is a naturally occurring bacteria used in the control of a variety of pests though its effects against LBAM appear to be limited. It is approved for use on organic produce. The primary concern with Bt is its potential environmental effects and effects against beneficial insect predators. Large-scale applications of Bt can have far-reaching ecological impacts. Bt can reduce dramatically the number and variety of moth and butterfly species, which in turn impacts birds and mammals that feed on caterpillars. In addition to negatively effecting food chain of wildlife, there is a potential for Bt to negatively affect the large populations of monarch butterflies that migrate and breed in Santa Cruz each year. While Bt is broadly reported to be non-toxic to bees, an international body of experts reported that mortality in bees has been observed after exposure to vegetatively growing Bt (UNEP 1999).

Bt is less toxic to mammals and shows fewer environmental effects than many synthetic insecticides. The EPA reports that Bt may give rise to secondary toxins that can affect non-target species. CDFA has announced intentions to treat residential areas with Bt.

b. Chlorpyrifos

CAS number: 2921-88-2

EPA: 738-F-01-006

Chlorpyrifos is a toxic crystalline organophosphate insecticide that inhibits acetylcholinesterase and is used to control insect pests. Product names include Dursban, Empire, and Lorsban. Cholinesterase inhibition in humans can result in over stimulation of the nervous system causing nausea, dizziness, confusion, and at very high exposures (e.g., accidents or major spills), respiratory paralysis and death. In 2001, EPA banned chlorpyrifos use in homes because of hazards to children. Approximately 2 million pounds of chlorpyrifos are used for agricultural purposes each year. The safety of chlorpyrifos has been questioned for more than a decade. In 1995, Dow Chemical was fined \$732,000 for not sending to the EPA reports it had received on 249 poisoning incidents associated with the product Dursban. In 2003, Dow agreed to pay \$2 million, the largest penalty ever in a pesticide case, to the state of New York, in response to a lawsuit filed by the Attorney General to end Dow's illegal advertising of Dursban as "safe". Concern over the safety of chlorpyrifos continues. On July 31st, 2007, a coalition of farm worker and advocacy groups filed a lawsuit against the EPA seeking to end agricultural use of chlorpyrifos. The suit claims that the continued use of chlorpyrifos poses an unnecessary risk to farm workers and their families (Earth Justice 2007). Additionally, the Natural Resources Defense Council (NRDC) and Pesticide Action Network of North America (PANNA) have formally petitioned the EPA to revoke all registrations and approvals for the use of chlorpyrifos. USDA has opposed this reclassification. Chlorpyrifos is not approved for home use except in ant and roach baits. As part of the LBAM eradication program, chlorpyrifos is currently required to be used in wholesale nurseries if a single sign of LBAM is found. In such cases, the entire acreage is required to be treated. This requirement presents a significant environmental health hazard as many of these nurseries are in residential areas, are along waterways, and are in close proximity to agricultural areas that utilize pollinators.

Chlorpyrifos is a neurotoxin and suspected endocrine disruptor that is classified by EPA as moderately toxic to humans (Class II). It predominantly affects the central nervous, cardiovascular, and respiratory systems and has been associated with asthma (AOEC Exposure Codes), reproductive and developmental toxicity. The OEHHA has prioritized chlorpyrifos to review as a potential reproductive toxin.

Chlorpyrifos is also a skin and eye irritant. While some organophosphates are readily absorbed through the skin, studies in humans suggest that skin absorption of chlorpyrifos is limited. Symptoms of acute exposure to organophosphate or cholinesterase-inhibiting compounds may include the following: numbness, tingling sensations, incoordination, headache, dizziness, tremor, nausea, abdominal cramps, sweating, blurred vision, difficulty breathing or respiratory

depression, and slow heartbeat. Very high doses may result in unconsciousness, incontinence, convulsions, and death.

Recent research indicates that children exposed to chlorpyrifos while in the womb have an increased risk of delays in mental and motor development at age 3 and an increased occurrence of pervasive developmental disorders such as ADHD (Whyatt et al. 2006). Another study demonstrated a correlation between prenatal chlorpyrifos exposure and lower weight and smaller head circumference of infants at birth (Whyatt et al. 2004).

Persons with respiratory ailments, recent exposure to cholinesterase inhibitors, cholinesterase impairment, or liver malfunction are at increased risk from exposure to chlorpyrifos. Some organophosphates may cause delayed symptoms beginning 1 to 4 weeks after an acute exposure, which may or may not have produced immediate symptoms. In such cases, numbness, tingling, weakness, and cramping may appear in the lower limbs and progress to incoordination and paralysis. Improvement may occur over months or years, and in some cases residual impairment will remain.

Repeated or prolonged exposure to organophosphates may result in the same effects as acute exposure including the delayed symptoms. Other effects reported in workers repeatedly exposed include impaired memory and concentration, disorientation, severe depressions, irritability, confusion, headache, speech difficulties, delayed reaction times, nightmares, sleepwalking, and drowsiness or insomnia. An influenza-like condition with headache, nausea, weakness, loss of appetite, and malaise has also been reported. A measurable change in plasma and red blood cell cholinesterase levels was seen in workers exposed to chlorpyrifos spray. Human volunteers who ingested 0.1 mg/kg/day of chlorpyrifos for 4 weeks showed significant plasma cholinesterase inhibition.

A body burden study conducted by the Centers for Disease Control and Prevention (CDC) found TCPy—a metabolite specific to chlorpyrifos—in the urine of 91% of people tested (CDC 2005). An independent analysis of the CDC data claims that Dow has contributed 80% of the chlorpyrifos body burden of people living in the US (PANNA 2004). A 2008 study found dramatic drops in the urinary levels of chlorpyrifos metabolites when children switched from conventional diets to diets consisting of higher amounts of organically cultivated foods (Lu et al. 2008).

Air monitoring studies conducted by the California Air Resources Board (CARB 1996) have documented chlorpyrifos in the air of California communities (Stein and White 1993). Analyses of the CARB data indicate that children living in areas of high chlorpyrifos use are often exposed to levels of the insecticide that exceed levels considered acceptable by the EPA (Kegley et al. 2003; Lee et al. 2004). Recent air monitoring studies in Washington and Lindsay, CA yielded comparable results (Dansereau et al. 2006; Kegley et al. 2006). Grower and pesticide industry groups have argued that the air levels documented in these studies are not high enough to cause significant exposure or adverse effects (Hansen 2007), but a follow-up biomonitoring study in Lindsay, CA has shown that people there have higher than normal chlorpyrifos levels in their bodies (Fischer 2007).

Chlorpyrifos is highly toxic to amphibians. A recent study by the United States Geological Survey (USGS) found that the main breakdown product in the environment, chlorpyrifos oxon, is even more toxic to amphibians than the primary compound (Science Daily 2007). When pure chlorpyrifos was fed to dogs for 2 years, increased liver weight occurred at 3.0 mg/kg/day. Signs of cholinesterase inhibition occurred at 1 mg/kg/day. Rats and mice given technical chlorpyrifos in the diet for 104 weeks showed no adverse effects other than cholinesterase inhibition. Two-year feeding studies using doses of 1 and 3 mg/kg/day of chlorpyrifos in rats showed moderate depression of cholinesterase. Cholinesterase levels recovered when the experimental feeding was discontinued. Identical results occurred in a 2-year feeding study with dogs. Occupationally, a single application of chlorpyrifos poses risks to small mammals, birds, fish and aquatic invertebrate species for nearly all registered outdoor uses.

Multiple applications increase the risks to wildlife and prolong exposures to toxic concentrations. Many nurseries in Santa Cruz County have been required to treat their entire acreage multiple times in only a few month-period.

Effects on birds: Chlorpyrifos is moderately to very highly toxic to birds. Its oral LD50 is 8.41 mg/kg in pheasants, 112 mg/kg in mallard ducks, 21.0 mg/kg in house sparrows, and 32 mg/kg in chickens. The LD50 for a granular product (15G) in bobwhite quail is 108 mg/kg. At 125 ppm, mallards laid significantly fewer eggs. There was no evidence of changes in weight gain, or in the number, weight, and quality of eggs produced by hens fed dietary levels of 50 ppm of chlorpyrifos.

Effects on bees: Chlorpyrifos is rated as highly toxic to bees, which means exposure can kill more than 1000 bees per hive daily. Exposure of hives to chlorpyrifos have resulted in 85 to 100% mortality in colonies (Bianu et al. year unknown).

Effects on aquatic organisms: Chlorpyrifos is very highly toxic to freshwater fish, aquatic invertebrates and estuarine and marine organisms. Cholinesterase inhibition was observed in acute toxicity tests of fish exposed to very low concentrations of this insecticide. Application of concentrations as low as 0.01 pounds of active ingredient per acre may cause fish and aquatic invertebrate deaths. Chlorpyrifos toxicity to fish may be related to water temperature. The 96-hour LC50 for chlorpyrifos is 0.009 mg/L in mature rainbow trout, 0.098 mg/L in lake trout, 0.806 mg/L in goldfish, 0.01 mg/L in bluegill, and 0.331 mg/L in fathead minnow. When fathead minnows were exposed to Dursban for a 200-day period during which they reproduced, the first generation of offspring had decreased survival and growth, as well as a significant number of deformities. This occurred at approximately 0.002 mg/L exposure for a 30-day period. Chlorpyrifos accumulates in the tissues of aquatic organisms. Studies involving continuous exposure of fish during the embryonic through fry stages have shown bioconcentration values of 58 to 5100. Due to its high acute toxicity and its persistence in sediments, chlorpyrifos may represent a hazard to sea bottom dwellers. Smaller organisms appear to be more sensitive than larger ones (EXTOXNET 1996).

Effects on other organisms: Aquatic and general agricultural uses of chlorpyrifos pose a serious hazard to wildlife and pollinators.

c. Permethrin

CAS Numbers:

- 52645-53-1 (mixed isomers)
- 54774-45-7 (cis-isomer)
- 51877-74-8 (trans-isomer)

Permethrin is one of a class of insecticides known as pyrethroids. It inhibits respiration in a manner similar to other neurotoxic drugs (Gassner et al. 1997 as cited by Cox 1998). Like other pyrethroids, permethrin kills insects by strongly exciting their nervous systems. In mammals it has been shown to cause a wide variety of neurotoxic symptoms including tremors, incoordination, elevated body temperature, increased aggressive behavior, and disruption of learning (Cox 1998). In an EPA summary of 17 medium-term and long-term laboratory studies that exposed test animals to permethrin, effects on the liver were noted at the "lowest effect level" in all of them (EPA 1997 as cited by Cox 1998).

Permethrin is classified as a "potential human carcinogen" by the EPA, and tests with human cells have shown it to be mutagenic. It is listed as a suspected endocrine disruptor, and both estrogen-like and antiandrogen-like effects have been observed in test animals. Endocrine disruptors are among the most insidious and damaging of pesticidal substances having been linked to breast and prostate cancer and a variety of reproductive disorders that can take decades to manifest and can effect multiple generations.

Studies have shown that pyrethroid exposure may be neurotoxic during development and that human newborns and children may be more sensitive to permethrin than adults. Children exposed to permethrin have developed immune-mediated respiratory and dermal irritation. Recent investigations of permethrin exposure of children have reported immunotoxic effects following exposure to pyrethroids, with increased incidence of anti-nuclear antibodies associated with autoimmune disease (EPA 2007).

Experiments with laboratory animals indicate that the immune system appears to be a sensitive target for permethrin activity. Ingestion of permethrin reduces the ability of T-lymphocytes to recognize and respond to foreign proteins (Cox 1998). Even small doses

equivalent to 1/100 of the LD₅₀, have been shown to inhibit T-lymphocytes by more than 40% (Cox 1998). Permethrin ingestion has also been shown to reduce the activity of natural killer cells by about 40 percent (Blaylock et al. as cited by Cox 1998).

Both the EPA and World Health Organizations have reported that permethrin increased the frequency of lung tumors in female mice, and increased the frequency of liver tumors in male and female mice (EPA 1997; WHO 1990 as cited by Cox 1998).

The toxic effects of permethrin are often greatly increased when combined with other chemicals. Several studies have linked a variety of health problems (commonly referred to as Gulf War Syndrome) reported by 30,000 veterans who served in the Persian Gulf War, with exposure to a combination of permethrin, the anti-nerve gas drug pyridostigmine bromide, and the insect repellent DEET.

Permethrin is highly toxic to a wide variety of animals including honeybees (and other beneficial insects), fish, aquatic insects, crayfish, and shrimp. It is especially toxic to cats. The potential toxicity of permethrin to beneficial insects is of specific concern with regards to the long term management of pests as a healthy ecosystem that fosters, not destroys, beneficial predators is the most effective, environmentally sound, and sustainable manner of controlling pests, including the LBAM.

In addition to toxic effects on beneficial insects needed for pollination of crops and a healthy ecosystem of natural predators, permethrin is highly toxic to both fresh water and estuarine aquatic organisms and can pose a serious threat to the Monterey Bay, a nationally protected marine sanctuary.

Studies have shown that most cats (96%) exposed to permethrin develop toxic effects, including excitability, twitching, tremors, convulsions, muscular weakness, respiratory distress, vomiting, diarrhea, hypersalivation, and death.

The State of California and the USDA intends to apply permethrin to pheromone traps and place tens of thousands of these traps in residential areas, the yards of private residents, schools, city parks, around day care centers, and on telephone poles throughout neighborhoods (3000 telephones per square mile). Dew, fog, mist, and rains will cause these toxins to leach into the surrounding areas, potentially acutely exposing families, playing children, and animals to this highly toxic compound and, in Monterey and Santa Cruz, eventually washing into the Monterey Bay, a protected marine sanctuary, as well as other estuaries in San Francisco, Marin, and other areas. Even small amounts of permethrin are classified as a "severe hazard to waters" under the European Administrative Regulation of Substances Hazardous to Water (Gestis Database).

Effects on bees: Permethrin is rated as highly toxic to bees, which means exposure can kill more than 1000 bees per hive daily.

d. Spinosad

CAS Numbers

- 131929-60-7 (Spinosyn A)
- 131929-63-0 (Spinosyn D)
- 168316-95-8 (used in WHO Acute Hazard list) (*PAN Database*)

Spinosad is a mixture of compounds formed from the fermentation of the soil organism *Saccharopolyspora spinosa*. The mixture is composed of approximately 10 related chemicals, with a variety of compounds derived from the fermentation process. Two closely related compounds, spinosyn A and spinosyn D, comprise about 88% of the composition of spinosad and are responsible for most of its insecticidal activity (JMPR 2001b). It kills insects through activation of the acetylcholine nervous system through nicotinic receptors. Continuous activation of motor neurons causes insects to die of exhaustion (USDA 2002).

The Dow Agrosiences products *Conserve* and *Entrust*, are the specific formulations recommended by the CDFA on its *Light Brown Apple Moth Approved Treatments for Nurseries and Host Crops* list. Both products contain spinosads (spinosyns) A & D as well as a variety of "inerts". *Conserve* includes propylene glycol (see separate toxicity review below) and *Entrust* includes porcelain clay, along with other unspecified inerts.

Spinosad is known to be highly toxic to honeybees as well as to beneficial parasitoid insects such as the *Trichogramma* wasp, which both provides biological protection against a host

of pests and acts as a food source for other organisms within the ecosystem. Spinosad is also highly toxic to oysters and other marine mollusks, moderately toxic to fish and marine invertebrates, and slightly toxic to birds. Adverse impacts against beneficial organisms are a particular concern; fresh sprays could kill honeybees and other parasitoids (Bret et al. 1997; Suh et al. 2000).

Spinosad is known to be highly toxic to honeybees as well as to beneficial parasitoid insects such as the *Trichogramma* wasp. It is also highly toxic to oysters and other marine mollusks.

Effects on bees: Spinosad is rated as highly toxic to bees, which means exposure can kill more than 1000 bees per hive daily. Spinosad was previously believed to be relatively safe to bees but a recent study by Canadian researchers demonstrated that bees in larval stage exposed to spinosad resulted in disruption of the ability of the adult bee to forage and that high degrees of exposure caused rapid colony collapse.

Observations of Bees in Santa Cruz After the Aerial Spray of Checkmate Pesticide Solution

Numerous Santa Cruz residents reported both direct bee die-offs and apparent disorientation of honeybees in gardens. Some reported gardening on Thursday November 8 the day of the evening spray and having their plants filled with honey bees. They then reported gardening the very next day in similar weather and the bees either gone or seeing thousands struggling on the ground and on plants, with one person reporting the bees flying into the plants and falling to the ground. Others reported no return of bees for 2-3 weeks after the spray and many people are reporting a lack of bees as we enter Spring time when there is typically an abundance of bees as the weather becomes warm and the flowers are in bloom. No formal data regarding population densities is available.

Epilogue to Bees

The relationship between wild pollinators and plant life spans a history of more than 400 million years (Kevan 1999). Pollination is as critical to the production of crops as water and sunlight and bees are the primary pollinators (Mussen 2004). If we destroy the bees there is no substitute when they are gone. If we destroy bees we not only destroy our food production but we destroy a significant portion of our world as insufficient pollination of wild plants can cause soil-holding and soil-enriching plants to die out turning lush areas into dustbowls (Bohart 1952). Many wild and ornamental plants require pollination to produce fruits and seeds that are a critical food supply to birds and wildlife. Bees are also visual indicators of the health of the environment. An abundance of bees reflects a healthy environment whereas a lack of bees often indicates the opposite (Grieg-Smith et al. 1994). Eradication programs that use chemical pesticides as the primary tools against pests are often wars against the environment as pesticides cause significant disruption of the normal balance between pest and predator, which in a healthy environment track each other. In a relatively infinitesimal period of time due to a variety of reasons including industrialization in general, loss of habitat, *Colony Collapse Disorder*, increased pesticide use, and mites most all wild bee colonies are gone. This leaves us with only hived bees to depend on for the entire pollination of plants. Thus we have a responsibility to preserve and protect the bees, if not for the integral role bees play in the ecosystem overall, then for the fact that our very survival and the survival of the world's food supply is dependent on bee colonies being protected and nurtured.

Before any further treatments are done, environmental assessments on the impact on all parts of the LBAM eradication treatment products should be conducted as to their impact on bees and potential contribution to *Colony Collapse Syndrome* and these studies should be based on long-term exposure of these materials. For bees these studies should include both physical effects of the microcapsules and sticky solution as well as systemic effects on all stages of the bees life.

Recommendations

- Discontinue the LBAM eradication program, changing to monitoring and control if necessary.
- Discontinue the use of chlorpyrifos in nurseries.
- Conduct environmental impact reviews for all components used in the LBAM eradication program, including the microcapsules, with a specific emphasis on the effects of each on pollinators, most notably honey bees.

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About the authors

Roy Upton is the Editor and Executive Director of the American Herbal Pharmacopoeia, Vice-President of the American Herbalists Guild, General Manager of Planetary Herbals and a long-term resident of Soquel, CA. Roy studied beekeeping at the University of Nevada.

Daniel Harder is the Director of the Arboretum at the University of California, Santa Cruz, has a doctorate in botany, had undergraduate studies in entomology, and was an avid beekeeper for more than 10 years.

Thomas Dadant has been a long time student and keeper of bees, the grandson of the bee keeping legend Pierre Camille Dadant who founded the oldest and largest bee supply company in the United States.