A Review of Acute Toxicity Studies Results on the Light Brown Apple Moth Pheromone Active Ingredient and Four LBAM Pheromone Products

Summary

The California Department of Food and Agriculture (CDFA), in cooperation with the U.S. Department of Agriculture (USDA), began the light brown apple moth (LBAM) eradication program in September 2007, with the aerial spraying of two pheromone products (Checkmate OLR-F and Checkmate LBAM-F) in Monterey and Santa Cruz counties. After the spraying occurred, a number of symptoms were reported from people in these areas. In response to these symptom reports, staff from the Department of Pesticide Regulation (DPR), the Office of Environmental Health Hazard Assessment (OEHHA), and the Department of Public Health (DPH) completed an evaluation of the available health and safety data related to these pheromone products[1], in addition to a summary of the symptoms reported[2]. These reports concluded that toxicology and exposure information indicated low potential for acute adverse health effects, and not enough information was available to determine if there was or was not a link between the symptoms and the pheromone applications. The possibility that some of the symptoms were caused by the application could not be ruled out.

In the next phase of safety evaluation for the LBAM eradication program, staff from DPR, OEHHA, and DPH reviewed and analyzed the results of a series of acute toxicity studies on four potential LBAM eradication products as well as the LBAM pheromone active ingredient (AI).

DPR requested a complete acute toxicity evaluation of the four products that USDA and CDFA have considered using in the next round of the LBAM eradication effort. The U.S. Environmental Protection Agency (U.S. EPA) has specific standards for acute toxicity testing for pesticides, which consist of a "six-pack" of tests: acute oral toxicity, acute inhalation toxicity, acute dermal toxicity, eye irritation, dermal irritation, and dermal sensitization. The products that were tested include Checkmate LBAM-F (sprayed aerially in Santa Cruz and Monterey counties in 2007), NoMate LBAM MEC, Splat LBAM, and Disrupt Bio-Flake LBAM. These studies were conducted by an experienced independent toxicology laboratory (Stillmeadow, Inc.), and were judged acceptable by U.S. EPA guidelines.

In addition, three studies on the pheromone active ingredient were voluntarily submitted by a manufacturer of the AI.

The results of these tests not only lay the foundation for understanding the toxicity of a product, but they also determine the product's toxicity category (I to IV) and the appropriate signal words to be used on the label. The results specify the required precautionary language, first-aid statements, personal protective equipment, hazard symbols, and re-entry intervals for the product as well.

The results of the acute toxicity tests on each of the four LBAM products indicated that they all had low potential for irritation, placing them in Category IV (Very Low Toxicity). Signs of toxicity were slight to none, and no abnormal clinical or necropsy observations were noted at the limit dose (highest dose recommended by U.S. EPA). Only the LBAM pheromone active ingredient reached Toxicity Category III (Low Toxicity) for the dermal irritation study. These test results indicate very low potential for acute toxicity from ingesting, breathing, or getting these products on the skin.

However, some difficulty arose in the analysis of the results of the dermal sensitization studies, as two different tests were available: the Buehler Guinea Pig Dermal Sensitization Study and the Local Lymph Node Assay. These two tests, both among U.S. EPA's preferred dermal sensitization studies, produced seemingly contradictory results. For Splat LBAM, NoMate LBAM MEC, and Checkmate LBAM-F, the Buehler Guinea Pig Dermal Sensitization studies were negative, while the Local Lymph Node Assays were positive. From a mechanistic standpoint, it is possible that the products are active in the initial phase of the sensitization process, which is what LLNA measures, but inactive in the later phases, what the guinea pig assay measures. While the results of the Buehler Guinea Pig Dermal Sensitization study on the LBAM pheromone active ingredient and Disrupt Bio-Flake LBAM were negative, further analysis of these two products as dermal sensitizers was not possible since LLNA tests were not available (active ingredient) or could not be conducted (Disrupt Bio-Flake).

In conclusion, the acute toxicity testing of several LBAM pheromone products indicates low acute toxicity to individuals who could have been exposed by ingesting, breathing, or getting the product on their skin. However, due to the positive results of the LLNA, we cannot dismiss the possibility that in sensitive individuals, contact with the particles could cause allergic-type responses, though the negative results of the Buehler assays do not provide a compelling argument for such a link. We find the results of the acute toxicity studies support our previous conclusion that we cannot definitively determine whether or not there is a link between the reported symptoms and the Checkmate applications and support our recommendation for enhancing the systems for symptoms reporting.

1. Consensus Statement on Human Health Aspects of the Aerial Application of Microencapsulated Pheromones to Combat the Light Brown Apple Moth

2. Summary of Symptom Reports in Areas of Aerial Pheromone Application for Management of the Light Brown Apple Moth in Monterey and Santa Cruz Counties September, October, and November 2007

Purpose and Overview

The purpose of this report is to provide recent information on the acute toxicity studies conducted on each of four pheromone-containing products that are being considered for use in the LBAM eradication effort. Even though aerial spraying of one or all of the

LBAM pheromone products is no longer planned for urban areas, the results of the acute toxicity studies are still relevant and important to inform the assessment of symptoms reported following past aerial applications, to inform future aerial applications in remote areas, and the evaluation of related products that may be used in ground-based LBAM eradication procedures.

This report is divided into four parts:

- The first part reviews the initial Consensus Statement that was released November 16, 2007, and the summary of the symptom complaints that was released April 10, 2008.
- The second part describes the methods used to assess acute toxicity and the results of those tests.
- The third part considers the relevance of these tests to the symptom reports associated with the Monterey and Santa Cruz Counties aerial applications in 2007.
- The fourth part, an addendum, discusses several issues related to the size of the Checkmate microcapsules.

This document represents an agreement among DPR, OEHHA, and DPH on the interpretation of acute toxicity studies results on LBAM pheromone active ingredient and four LBAM pheromone-containing products.

Review of Past Joint Activities

In September 2007, CDFA, in cooperation with USDA, initiated a program to eradicate the LBAM in Monterey and Santa Cruz Counties, which involved the aerial application of two products containing microencapsulated pheromones (Checkmate OLR-F and Checkmate LBAM-F). Subsequent to the applications, almost 500 symptom reports were received from people in the treatment areas. Staff from DPR and OEHHA, in cooperation with DPH staff, conducted an evaluation of the available health and safety data related to the pheromone products used. That report, "Consensus Statement on Human Health Aspects of the Aerial Application of Microencapsulated Pheromones to Combat the Light Brown Apple Moth," (the consensus statement) was released November 16, 2007. While the toxicology and exposure information suggested a low probability of adverse health effects, the possibility that some of the reported symptoms were caused by the application could not be ruled out. The consensus statement noted:

> *"A study on a chemical similar to one of the active ingredients in the LBAM pheromone does indicate some potential for limited dermal sensitization."*
>
> *"Most reported symptoms are consistent with inhalation of a nonspecific irritant material, but because they are also consistent with other possible causes, it is not possible to confirm the symptoms are or are not due to the application of Checkmate."*

> *"Based on the available toxicological information on the Checkmate product, some of the reported health effects such as eye, skin, or respiratory irritation could be consistent with inhalation of a sufficient amount of the applied material. But because the measurements confirm the application rate was extremely low, it is likely that exposure occurred at levels below those that would be expected to result in health effects. However, because not all health effects can be predicted and because the general population includes susceptible populations, such as children, the elderly, and those with chronic diseases, we cannot provide a definitive cause for their symptoms."*

In addition to the above review, staff from DPR, OEHHA, and DPH prepared a summary of the symptom reports, and assessed the relationship between the symptoms and the aerial application of Checkmate. The report, "Summary of Symptom Reports in Areas of Aerial Pheromone Application for Management of the Light Brown Apple Moth in Monterey and Santa Cruz Counties September, October, and November 2007," (the summary of symptom reports) concluded that the majority of the reports did not contain enough information to determine whether there was a link between symptoms and application. The report was released April 10, 2008 and noted:

> *"In this report, respiratory symptoms (such as cough, shortness of breath, runny nose, upper respiratory irritation/pain, and wheezing) were prominent, reported by 321 (70%) of the 463 individuals who reported symptoms. Among those who sought medical attention, 62 of 74 (84%) reported respiratory symptoms. PIR reports indicated seven diagnoses of asthma exacerbation, two of asthma, and one of reactive airway disease. The remaining PIRs indicated many of the respiratory symptoms cited above, along with headaches and diarrhea."*

> *"More than 90 percent of the 463 symptom reports do not contain adequate information for us to determine whether or not there is a link between the reported symptoms and the Checkmate applications. . . In addition, the fact that there is no diagnostic test to confirm pheromone exposure, the high background symptom reporting rate and the very low pheromone application rates make it very difficult to determine with any degree of certainty if the symptoms were caused by the pheromone formulation."*

In preparation for the eradication effort in the summer of 2008, USDA and CDFA considered four LBAM pheromone products for potential aerial application. These products were Checkmate LBAM-F (product sprayed aerially in Santa Cruz and Monterey counties), NoMate LBAM MEC, Splat LBAM, and Disrupt Bio-Flake LBAM (Table 1). DPR requested a full set of acute toxicity studies on each of these product formulations. USDA, with advice from U.S. EPA's Office of Pesticide Programs (OPP) Registration Division, contracted with an experienced independent toxicology laboratory (Stillmeadow, Inc.) to conduct the relevant studies.

Table 1- Description of the Four Tested LBAM Products

Product	Characteristics
Checkmate LBAM-F	LBAM pheromone in microcapsule shell, aqueous suspension, previously applied
NoMate LBAM MEC	LBAM pheromone in microcapsule shell, aqueous suspension
Splat LBAM	LBAM pheromone in an amorphous sticky polymer, size of drops depends on application characteristics
Disrupt Bio-Flake LBAM	LBAM pheromone in polymer flakes, approximately 1/8 inch square by 1/16 inch thick, applied with a sticking agent

Subsequent to the initiation of these studies, CDFA announced on June 19, 2008, that sterile moth technology had advanced sufficiently that they would be able to produce sufficient sterile LBAMs to conduct an insect release program. This new alternative would eliminate the need for aerial spraying over urban areas. This approach involves the production and release of sterile male moths to cause population collapse. The use of aerial spraying of pheromone products would be reserved only for remote areas and agricultural sites inaccessible by ground vehicles. CDFA also announced plans for the ground-based application of additional pesticides.

Even though aerial spraying of one or all of the above LBAM pheromone products is no longer under consideration for urban areas, the results of the acute toxicity studies are still relevant. The two pesticide regulatory agencies, U.S. EPA and DPR, reviewed the acute toxicity studies and judged all of them to be acceptable

Concurrent with the conduct and submission of the acute toxicity studies on the four formulated products, the manufacturer of the pheromone active ingredient (Bedoukian Research Inc.) submitted an acute oral study, a dermal irritation study, and a Buehler dermal sensitization study on the LBAM pheromone active ingredients. LBAM pheromone mixtures similar to those submitted for testing by Bedoukian Research Inc. are in all four pheromone products. DPR did not request these studies, but Bedoukian Research submitted them voluntarily to assist in the overall toxicity evaluation. An independent contract laboratory (MB Research Laboratories) conducted these studies.

Acute Toxicity Studies

Procedures for Studying Acute Toxicity in Pesticide Products

U.S. EPA establishes the data requirements for pesticides, including the toxicity data requirements (Code of Federal Regulations-CFR 40, Subpart F-Toxicology, 158.500). The acute toxicity study requirements are generally the same for all pesticide products. While these studies are aimed at acute or short-term toxicity and do not directly measure effects that might occur

from intermediate or long-term exposures, they do provide valuable information that is relevant to these exposures. The acute toxicity studies form the basis for the understanding of the toxicity of a compound in question. In addition to being used in health evaluations, the results of the studies form the basis for the selection of the appropriate signal words, precautionary language, first-aid statements, personal protective equipment, hazard symbols, and reentry intervals that are required on the product label. The U.S. EPA-required acute toxicity studies are: acute oral toxicity, acute dermal toxicity, acute inhalation toxicity, primary eye irritation, primary dermal irritation, and dermal sensitization. These studies are conducted using laboratory animals. These six studies are sometimes informally referred to as the "six-pack." U.S. EPA has published guidelines for the conduct of the various required studies, including these acute toxicity studies. In addition, all toxicity studies must be conducted following the federal Good Laboratory Practices (GLP) standards. GLP is a set of principles and requirements, under which studies are planned, performed, monitored, recorded, reported, archived, and audited. The GLP framework ensures the quality and integrity of submitted test data.

Following the conduct of a study, the study sponsor submits a study report to the relevant regulatory agency. The study report includes the study protocol, documentation of the conduct of the study, and the individual study data. The report is reviewed in detail by the regulatory agency. Reviewers pay attention to questions such as whether enough animals were used, the appropriate dose levels were used, sufficient data were included in the study reports, adequate histopathology was conducted, the tests material was adequately characterized, etc.

Descriptions of the six studies are included below:

Acute oral toxicity study

The preferred test species is the rat and at least five animals are used at each dose level. The material is given in a single dose orally. After completion of the test in one sex, at least one group of five animals in the other sex is used to establish that the animals of this sex are not markedly more sensitive to the test substance. An observation period of about 14 days follows the exposure during which the signs of toxicity may appear. Animals are observed several times on the day of dosing and once daily thereafter, for abnormal clinical/behavioral signs (e.g., respiratory effects such as labored breathing; evaluation of skin, fur, eyes, and mucus membranes; circulatory effects; autonomic effects such as salivation; central nervous system effects including tremors and convulsions; changes to the levels of motor activity, gait, and posture, reactivity to handling or sensory stimuli; or unusual behavior). Any abnormal signs are recorded. Observations also include such things as body weight and food consumption. The various observations are recorded. At the end of the observation period, the test animals are killed and undergo a gross examination.

An oral LD_{50} (the dose that causes death in 50 percent of the treated animals) of less than 50 milligrams of pesticide per kilogram of an animal's body weight (mg/kg) would indicate High Toxicity, place the material in Category I (highest category of toxicity) for oral toxicity, and would require a signal word of "Danger" as well as specific precautionary language on the label.

At the other end of the spectrum, an oral LD_{50} of greater than 5,000 mg/kg would indicate Very Low Toxicity, would place the material in Category IV for oral toxicity, and would require a signal word of "Caution" on the label with no precautionary statement. For each study type, there is what is referred to as a limit dose. This is a very high dose level and U.S. EPA does not consider going above it capable of providing any relevant toxicity information. For acute oral toxicity, the level is 5,000 mg/kg.

Acute inhalation toxicity study

This study is similar in many respects to the conduct of the oral study, except that the inhalation route of exposure is used. Again, the rat is the preferred test species. A great deal of attention is devoted to generating and characterizing the test material or aerosol. During the development of the generating system, a particle size analysis is performed. Animals are generally exposed to the test aerosol for a period of 4 hours. Following exposure, there is an observation period of 14 days. Animals are observed several times on the day of dosing and once daily thereafter, for abnormal clinical/behavioral signs (same as for acute oral toxicity study above). Any abnormal signs are recorded. A series of doses or test concentrations are used. For the inhalation study, the limit dose is 2 milligrams of pesticide per liter of air (mg/l) for 4 hours. When it is not possible to achieve this concentration due to physical or chemical properties of the test substance, the maximum attainable concentration is employed. In this case, the study report should contain a detailed explanation of why a higher concentration could not be attained. In some cases, the product may not be in a respirable form and it may not be possible to generate a respirable test aerosol. This could happen with a material that consists of very large particles that are too large to be inhaled into the deep portions of the lung and are not amenable to being ground into respirable form for testing. In these cases, it would not be possible to conduct a scientifically relevant inhalation study, and the study report or data submission would contain a detailed explanation of the limitations on generating a test aerosol and a characterization of the product showing that the product as used would not be respirable. An inhalation LC_{50} (the concentration in air that causes death in 50 percent of the treated animals) of less than 0.05 mg/l would indicate Category I for inhalation toxicity, while an LC_{50} of greater than 2 mg/l would place the material in toxicity Category IV.

Acute dermal toxicity

This study evaluates signs of systemic toxicity following dermal exposure, which includes adverse effects on the body beyond the site of exposure. The rabbit is the preferred test species because of its size, ease of handling, and skin permeability. At least five animals per sex per dose are used. The test material is moistened if it is a solid, usually with water, placed over a shaved area on the skin, and held in contact with the skin by a gauze patch. After a 24-hour period, the residual test material is removed. A series of dose levels are used and a 14-day observation period is recommended. Animals are observed several times on the day of dosing and once daily thereafter, for abnormal clinical/behavioral signs (same as for acute oral toxicity study above). Any abnormal signs are recorded. The limit dose is in the range of 2,000 to 5,000 mg/kg. A

dermal LD_{50} of less than 200 mg/kg would place the material in Category I for dermal toxicity, while an LD_{50} of greater than 5,000 mg/kg, would place the material in Category IV.

Eye irritation study

The test material is applied in a single dose to one of the eyes in each of several test animals, and not washed out for 24 hours. The degree of irritation is evaluated, scored at specified intervals, and described to provide a complete evaluation of the effects and to determine the specific toxicity category. The preferred test species is the rabbit. Following application, the animals are observed for at least 72 hours, and up to 21 days depending on the appearance and resolution of any irritation. Corrosiveness (a scientific term that refers to the destruction of living tissue by a foreign substance) or corneal opacity that is not reversed after 7 days would indicate a Category I classification, while no irritation would be indicative of a Category IV classification. If it is already known that the material is corrosive, this study is not required and is not appropriate.

Dermal irritation study

The test substance is applied to the skin of several test animals with each animal serving as its own control. The degree of irritation is read and scored to provide a complete evaluation of effects and to determine the toxicity category. The preferred test animal is the rabbit. The test material is moistened if it is a solid, placed on an area of shaved skin, and covered by a gauze patch for 4 hours. At the end of the exposure period, that patch is removed along with any residual material. After removal of the patch, the animals are examined for signs of redness or swelling and the responses scored at several specified intervals up to 72 hours. The duration of the observation period (up to 14 days) should be sufficient to fully evaluate the reversibility or irreversibility of any effects. Corrosiveness would indicate a Category I classification, while mild or slight irritation at 72 hours would be indicative of a Category IV classification. Like the eye irritation study, if it is already known that the material is corrosive, this study is not appropriate.

Dermal sensitization study

The purpose of the dermal sensitization test is to identify substances with the potential to cause "skin sensitization" or allergic contact dermatitis. This test examines a chemical's ability to cause a skin reaction or allergic-type response that involves the immune system. In the case of the other five studies, the guidelines specify a single study protocol with a preferred animal species. With the dermal sensitization study, U.S. EPA accepts several different protocols, with three that are generally preferred. The three protocols that U.S. EPA specifies in its guidelines are the Local Lymph Node Assay (LLNA), the Guinea-Pig Maximization Test (GPMT), and the Buehler test. If the results of one of the studies indicate sensitization, a specific toxicity category is not assigned, but a warning statement to the effect that repeated exposure could lead to dermal sensitization is generally required on the label. The wording of this statement may vary.

With the Buehler and GPMT methods, a total of 15 male and 15 female guinea pigs are dermally exposed to a test substance. Five of each sex are placed in the control group, while 10 of each

sex are placed in the treatment group. There is a rest period of 10 to 14 days (the induction period) during which signs of an immune response, (e.g., observation of redness, or inflammation at the site of contact) may develop. The animals are then given a repeat or "challenge" exposure. This second exposure determines whether the initial exposure caused the animal to become "sensitized" to the test substance. Examining the dermal reaction to the challenge exposure and comparing this reaction to the one following the initial exposure determines the presence of sensitization, or an immune system based response. The GPMT differs from the Buehler Assay in that the GPMT utilizes the injection of an adjuvant (a nonspecific chemical agent that stimulates the immune system) to induce sensitization. These studies focus more on the elicitation phase or expression steps (one of the last steps) of the sensitization process than does the LLNA.

The LLNA is based on the principle that skin sensitizers cause lymphocytes (a type of white blood cell in the immune system) to multiply at an abnormally high rate in the lymph nodes close to the site of chemical application. Therefore, this assay focuses on the initiation phase (one of the first steps) of the sensitization process. A minimum of five mice are used at each dose level. The test material is applied to the back of both ears for each of three consecutive days. On day 6, radioactive thymidine is injected into each mouse. The mice are killed five hours later and the appropriate lymph nodes are removed. The rate of thymidine incorporation into the lymph nodes indicates the rate of lymphocyte proliferation.

While the GPMT and Buehler tests have a long history of use, the LLNA has been more recently validated and adopted. In 1998, the federal Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) evaluated the status of the LLNA as an alternative to the guinea pig assays and recommended the LLNA as a valid study for most situations. However, based on the lack of available data, the ICCVAM recommended that the LLNA not be used in a few situations, including for "mixtures." A mixture refers to a substance containing more than one chemical ingredient. On December 11, 2001, the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel (SAP) met at the request of U.S. EPA to provide advice on the applicability of the LLNA as a stand-alone assay for dermal sensitization and found that the LLNA had been validated and shown to perform as well as the traditional guinea pig assays for prediction of human sensitization for a broad range of chemicals, including pesticides, industrial chemicals, and a limited number of pharmaceuticals. U.S. EPA accepted the LLNA as a stand-alone assay for pesticides.

While the LLNA and guinea pig assays are now considered equivalent in their ability to predict human dermal sensitization, sometimes their results do not agree, and all three ***can*** give false positive and false negative results pertaining to the effects seen in humans. The ICCVAM met in 2007 and released in January 18, 2008, a "Draft Updated Assessment of the Validity of the LLNA for Mixtures, Metals, and Aqueous Solutions." This assessment concluded that there were insufficient data on mixtures tested by both LLNA and guinea pig assays, and of those limited data results, there was relatively poor concordance between the two assays. As a result, the use of the LLNA for mixtures was neither validated nor invalidated in this assessment. However, the LLNA is currently one of the three dermal sensitization assays preferred by the U.S. EPA for

pesticide products. In addition, the European Union (EU) and the Organization for Economic Cooperation and Development (OECD) specify a preference for the LLNA over the guinea pig assays (primarily for animal welfare considerations).

Results of the Acute Toxicity Studies
The following section discusses the results of the acute toxicity studies for the four LBAM pheromone products and the LBAM pheromone active ingredient. Additionally, a summary of these results is provided in Table 2 at the end of the section.

Disrupt Bio-Flake LBAM

The Disrupt Bio-Flake pellets were too large for the conduct of the acute oral or inhalation toxicity studies. Two procedures were attempted to grind the flakes into smaller particles that would permit the conduct of the studies; however, neither grinding method was successful so these studies could not be conducted. The dermal toxicity study was conducted at the limit dose of 5050 mg/kg and showed no significant clinical signs. The dermal irritation study did not demonstrate any irritation, and the eye irritation study showed only slight irritation, both indicating Toxicity Category IV hazards. The Buehler Guinea Pig Dermal Sensitization Study indicated that the material is not a dermal sensitizer. A LLNA dermal sensitization study was undertaken to assess the test article's potential to be a skin sensitizer in that assay; however, the physical and chemical properties of the material did not permit the performance of the test.

Checkmate LBAM-F

The acute oral, inhalation, and dermal toxicity studies were conducted at the limit doses (5,000 mg/kg, 2 mg/l and 5050 mg/kg, respectively). The particle aerosol that the animals were exposed to had a reported median (in terms of mass) diameter of 2.2 micrometers. The studies showed no significant abnormal clinical or necropsy signs and all clearly indicated Toxicity Category IV classification. The dermal irritation and eye irritation studies showed only slight irritation, both indicating Toxicity Category IV. The Buehler Guinea Pig Dermal Sensitization Study did not indicate dermal sensitization. The LLNA, on the other hand, showed lymphocyte proliferation indicating the product's potential as a dermal sensitizer.

NoMate LBAM MEC

The acute oral, inhalation, and dermal toxicity studies were conducted at the limit doses (5,000 mg/kg, 2 mg/L, and 5050 mg/kg respectively). The studies showed no significant abnormal clinical or necropsy signs and showed minimal to no clinical or necropsy signs of toxicity, and all clearly indicated Toxicity Category IV. The eye irritation study showed only slight irritation and the dermal irritation study showed no signs of irritation, both indicating Toxicity Category IV. The Buehler Guinea Pig Dermal Sensitization

Study did not indicate dermal sensitization. The LLNA, on the other hand, showed lymphocyte proliferation indicating the product's potential as a dermal sensitizer.

Splat LBAM

The acute oral, inhalation, and dermal toxicity studies were conducted at the limit doses (5,000 mg/kg, 2 mg/L, and 5050 mg/kg respectively). The studies showed no significant abnormal clinical or necropsy signs and all indicated Toxicity Category IV. The dermal irritation and eye irritation studies showed only slight irritation, both indicating Toxicity Category IV. The Buehler guinea pig dermal sensitization study indicated that the material is not a dermal sensitizer. The LLNA, on the other hand, showed lymphocyte proliferation indicating the product's potential as a dermal sensitizer.

LBAM Pheromone Active Ingredient (manufactured by Bedoukian Research, Inc.)

The acute oral toxicity study was conducted at the limit dose (5,000 mg/kg) and indicated Toxicity Category IV with no significant clinical signs. The dermal irritation study produced moderate irritation indicative of Toxicity Category III. The Buehler dermal sensitization study did not indicate dermal sensitization. An LLNA was not conducted on the LBAM pheromone active ingredient; precluding further comparisons with the study result on the four LBAM formulated products.

Table 2- Summary of the Acute Toxicity Study Results[a]

Product	Oral (5000 mg/kg)	Inhalation (2 mg/L for 4 hours)	Dermal Toxicity (5050 mg/kg)	Dermal Irritation	Eye Irritation	Dermal Sensitization	
						Beuhler	LLNA
Checkmate LBAM-F	Very low toxicity Category IV[a]	Very low toxicity Category IV	Very low toxicity Category IV	Slight Category IV	Slight Category IV	−	+
NoMate LBAM MEC	Very low toxicity Category IV	Very low toxicity Category IV	Very low toxicity Category IV	None Category IV	Slight Category IV	−	+
Splat LBAM	Very low toxicity Category IV	Very low toxicity Category IV	Very low toxicity Category IV	Slight Category IV	Slight Category IV	−	+
Disrupt Bio-Flake LBAM	NA	NA	Very low toxicity Category IV	None Category IV	Slight Category IV	−	NA
LBAM Pheromone AI	Very low toxicity Category IV	NA	NA	Moderate Category III[b]	NA	−	NA

NA – not applicable. Test was not performed.

a-Based on the results of a study, a pesticide is assigned a Toxicity Category. Category I indicates the highest toxicity and Category IV the lowest toxicity. Category IV would lead to a signal word of "Caution" on the label with no precautionary statement.
b- Category III for dermal irritation would lead to a signal word of "Caution" on the label along with a precautionary statement for dermal irritation.

Discussion

The results of the acute toxicity studies, with the exception of the dermal sensitization studies, clearly indicate very low acute toxicity (Toxicity Category IV) with no remarkable clinical or necropsy signs. The LLNA studies were adequately conducted and indicate the potential for dermal sensitization under the conditions of the assay. The Buehler assays were conducted at the same contract laboratory (with the exception of the assay on the LBAM pheromone active ingredient) and were likewise adequately conducted, but did not show dermal sensitization.

These seemingly different results are not necessarily contradictory, given the deliberations and conclusions of the various advisory panels previously discussed. From a mechanistic standpoint, it is conceivable (although specific supporting data are not available) that the pheromone products could be active in an initial phase of the sensitization process (as measured by the LLNA) but inactive in later phases (as measured by the guinea pig assays). In other words, it is possible that the pheromone products could cause the reactions in the lymphatic system observed in the LLNA tests without causing actual symptoms (skin rashes, etc.) in the test animals.

In the absence of additional data, the health-protective approach is to treat the products as potential dermal sensitizers, meaning that they have the potential to cause allergic type reactions from skin contact. There have been various suggestions in the scientific literature to use the LLNA as a screen for potential respiratory sensitization (hypersensitivity of the airways, e.g., coughing, wheezing, asthma); however, this use or application has not been validated. While we cannot view the LLNA tests as evidence that exposure to the pheromone products can cause respiratory sensitization, this possibility cannot be ruled out.

There are a number of sources of uncertainty that have to be considered when extrapolating the results of animal studies to predict or explain possible effects in people. These include:

- The small number of animals used in the studies compared to the large number of people who could be exposed to an aerially applied material.

- Multiple exposure routes for people compared to single exposure routes (oral, dermal, or inhalation) in animal studies.

- The potential differences in sensitivity between the species used in the laboratory studies relative to people (though the specific animal species in each assay are chosen for their high sensitivity to reflect sensitive individuals).

- The genetically similar nature of laboratory animals compared to a genetically diverse human population.

- The high exposures and direct applications of materials used in these assays compared to the low exposures that resulted from aerial applications.

- The lack of agreement between the results of the LLNA and Buehler tests for dermal sensitization and the applicability of the LLNA for testing mixtures.

- The lack of an LLNA for the pheromone active ingredient, which could help address whether the active ingredient or the inert ingredients in the products resulted in the positive LLNA results.

- The relevance of the results of the LLNA dermal sensitization study to human dermal or respiratory sensitization.

- The paucity of toxicity data following long-term exposures. The conclusions here are based upon a single exposure or a limited number of short-term exposures separated by weeks or months.

Relevance/Implications of the Results of the Acute Toxicity Studies to the Summary Symptom Reports

Respiratory symptoms were prominent in the reports provided by residents in the areas treated with Checkmate pheromone products. Most of these symptoms were consistent with exposure to an irritant. A smaller number of cases of asthma exacerbation and reactive airway disease were reported; in general these conditions may be associated with exposure to a "sensitizer" or allergen. In addition to respiratory symptoms, some residents in treated areas reported dermal symptoms, such as skin irritation and pain, itching, and rash.

The negative Beuhler test results for the pheromone product Checkmate LBAM-F and the pheromone active ingredient support the product's low potential for dermal sensitization (immune system-mediated skin reaction). On the other hand, the positive LLNA result in Checkmate LBAM-F suggests a potential to cause this type of allergic reaction that cannot be dismissed. Since the LLNA assay considers a different aspect of the immune response, we cannot exclude the possibility that one or more ingredients in the LBAM product could cause an allergic response in sensitive individuals. However, the very low application rates would decrease the potential for such reactions.

In conclusion, the acute toxicity testing of several LBAM pheromone products indicates low acute toxicity to individuals who could have been exposed by ingesting, breathing, or getting the product on their skin. However, due to the positive results of the LLNA, we cannot dismiss the possibility that in sensitive individuals, contact with the particles

could cause allergic-type responses, though the negative results of the Buehler assays do not provide a compelling argument for such a link. We find the results of the acute toxicity studies support our previous conclusion that we cannot definitively determine whether or not there is a link between the reported symptoms and the Checkmate applications and support our recommendation for enhancing the systems for symptoms reporting.

Addendum: Size of Checkmate Microcapsules

At the time the 2007 consensus statement was prepared, information provided by the manufacturer indicated that the microcapsule particles were large by inhalation standards (exceeding 25 micrometers in diameter) and unable to reach the deep lung. Later analyses of the particle-size distribution of the Checkmate product indicated that by count almost 50 percent of the particles were smaller than 10 micrometers in physical diameter. Particles smaller than 10 micrometers are capable of reaching the deep lung, which has led to questions as to whether the consensus statement conclusions should be revised to reflect the updated information on particle size.

When inhaled, a majority of the Checkmate particles are likely to be deposited in the upper lung. In a matter of days, they are moved by the mucociliary "escalator" to the throat and swallowed. The microcapsules are anticipated to be quite stable and able to pass through the gastrointestinal tract without change. A small percentage of the Checkmate particles may reach the alveolar or pulmonary region (deeper lung) and stay there for a longer period of time, many months or even longer. If that happens, the polyurea shell of the microcapsules can either stay intact or degrade and release its contents.

The conventional way to evaluate the health effects of airborne particles is by measuring their weight. For instance, the federal and the California State 24-hour average ambient air standards for PM_{10} (airborne particles smaller than 10 micrograms) are 150 micrograms of airborne particles per cubic meter of air ($\mu g/m^{3)}$ and 50 $\mu g/m^3$, respectively. Both of these standards are based on the weight of the sub-10 micron particles measured in a cubic meter of air, not the number of particles. While almost half the Checkmate particles were smaller than 10 micrometers, these particles accounted for only about 1 percent of the total weight of the Checkmate product. No particles were smaller than 4.5 micrometers in physical diameter.

A DPR study and analysis found that 3 ounces (85 grams) of the Checkmate formulation were deposited per acre within the aerial-application areas during the 2007 applications in Monterey and Santa Cruz counties. Only 1 percent of this amount (0.85 grams) consisted of particles smaller than 10 micrometers. The airborne concentration of Checkmate particles smaller than 10 micrometers would have been less than the state PM10 standard of 50 micrograms per cubic meter of air.

For this reason, the new information on the size of the Checkmate particles, along with the results of the acute toxicity tests, do not change the central conclusion of the consensus statement, which said: “Taken together, the toxicity data on the pheromones and on microencapsulated products suggest the possibility that exposure to a sufficient amount of airborne Checkmate microcapsule particles could result in some level of eye, skin, or respiratory irritation. However, as the product is diluted and applied over a large area, the degree of exposure as well as the potential for irritation should decrease significantly.”