Commentary On The 6-pack Toxicology Testing For LBAM Eradication Pesticide Products

Ann Haiden, D.O.

November 25, 2008

In 2007, the residents of Monterey and Santa Cruz counties were aerially sprayed with Checkmate OLR-F and LBAM-F pheromone pesticide. Over 600 health complaints ensued, but after reviewing the complaints, OEHHA (The Office of Environmental Health Hazard Assessment) determined that they could not link the complaints to the spray because of the inadequacy of the data. Subsequently, a "6-pack" of tests on the pheromone pesticide product were performed. These results have finally been reported by the CDPR, OEHHA and CDPH. Individuals with health complaints and reporting clinicians still have not been interviewed.

The acute effect six-pack tests performed do not provide what is needed to test for the hazards of products meant to be used in a timed-release, chronic and repetitive fashion on a genetically diverse group of people with multiple pre-existing health conditions. The authors of a consensus statement released on November 4, 2008, *A Review of Acute Toxicity Studies Results on the Light Brown Apple Moth Pheromone Active Ingredient and Four LBAM Pheromone Products*, admit to some of these deficiencies.[1] Yet, the tests do bring to light real possible dangers.

The consensus authors bring forth several potential drawbacks of the results: the small number of animals, multiple exposure routes for people as compared to in the tests, potential differences in sensitivity between people and the animals, the genetically similar nature of laboratory animals compared to the genetic diversity of humans, the lack of LLNA testing for the active ingredient (which would help determine if the active or inert ingredients are causing the result) and the lack of toxicity data for long-term exposure. Although the test exposures used higher than normal exposure, it gives no information about chronic timed-release lower dose exposure. According to the authors, "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." And, "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."

Furthermore, an emphasis was placed upon the active ingredient, without recognition of the importance of inert ingredients. Testing on the isolated pheromone chemicals was incomplete. The currently most widely used application of the pesticide in the community, the twist tie, was not tested at all.

There were worrisome findings that require further explanation and investigation, such as abnormal organs, one death and consistent evidence of skin sensitization related lymph node activation in the test animals. This should lead to precaution in this rush to use widespread LBAM eradication products in communities.

In addition to the toxicological findings, dispersal studies show the pheromone pesticide was not dispersed evenly in the application mixture itself. Ground areas of exposure had vastly different pheromone pesticide concentrations. And, there was a large amount of drift of the applied mixture, resulting in uneven application and to exposure of buffer zones to pesticide. The result was that areas that were not meant to be sprayed, were sprayed. And, some people were sprayed with higher doses than planned, while others were sprayed with lower doses.[2]

Findings summary

Product was tested by Stillmeadow, Inc. for acute eye irritation, acute oral toxicity, acute inhalation toxicity, acute dermal irritation, skin sensitization via the local lymph node assay (LLNA) and skin sensitization in guinea pigs.[3] The pheromone "active ingredient" chemicals were tested separately by the manufacturer but only for acute oral and eye toxicity and dermal irritation. The LBAM-F spray, NoMate LBAM MEC, Splat LBAM and Disrupt Bio-Flake LBAM were the products tested. All of the products that were tested for lymph node reactivity were positive (LBAM-F spray, NoMate and Splat).

The encapsulated LBAM-F product, when tested for acute inhalation toxicity with a single 4-hour exposure, revealed abnormal organs in 50% of the animals at necropsy, described as a combination of dark red livers, pale lungs or both. One animal lost weight. Pale lungs can occur due to decreased uptake or destruction of hemoglobin.

In the acute dermal toxicity test, which exposed the skin of animals to LBAM-F product for 24 hours under an occlusive wrap, one animal died the first day after exposure. It had abnormal dark red lungs, liver and spleen. Dark red lungs can be associated with pulmonary congestion. Two other animals lost weight. Since there were only 10 animals in this test, the mortality rate was 10%. Even though the tests are meant to look for death of 50% of the animals, it remains unknown why one animal actually did die on day one. There was no reason or hypothesis given for the abnormal organs or death in either of these tests.

The local lymph node assay in mice (LLNA), a test for allergic skin sensitization, tested 3 groups of 5 animals each. One group at 25% concentration, one group at 50% concentration and one at 100% concentration. The product is applied to the ears once per day for 3 days, then a radiotracer substance is injected which is taken up in the lymph nodes, more or less, depending on how much lymphocyte proliferation there is in the lymph node. The lymph nodes are then tested to see how much tracer was taken up. This is then compared to the results in animal groups that had been given either a placebo-like substance or a substance that is known to give a strong positive reaction. One animal lost weight. The 50% and 100% concentration groups all had strong enough reactions of increased lymphocyte proliferation to qualify as positive. The 25% group also had increase in reactivity but not enough to meet positive criteria.

Reactions were not confined to the aerosolized LBAM-F formulation but across the spectrum of application products. The LLNA test was positive in all of the products it was performed on. NoMate acute toxicity testing resulted in piloerection, a sign of sympathetic nervous system stress response. The isolated pheromone testing had dermal

erythema lasting up to 72 hours and delayed contact sensitivity testing (Buehler) resulted in 2 of 3 animals having diarrhea and one with anogenital soiling.

A number of the products consistently showed eye and dermal irritation. That is clearly consistent with the complaints of the Monterey and Santa Cruz residents who were sprayed. There was no other environmental presence at that time that could have affected such a broad group of people within a specific geographic area.

Discussion

The LBAM eradication products are designed to be time released and dispersed in various vehicles. Some of the products, however, proved hard to solublize for testing and, in the case of LBAM-F, the testing was done with encapsulated product. Therefore, we do not know the full effect of the LBAM-F chemical ingredients, which would only become fully apparent over time. If the testing had been done with unencapsulated product, then the effect of the capsule would not be obvious.

We do not know why 50% of the animals exposed via inhalation to the LBAM-F spray had abnormal organs. The authors only report that "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."

We do not know about respiratory system lymph node reactivity, inflammatory cascades, any effect on Clara cells, cardio-pulmonary effects, oxidative stress, anti-oxidant consumption, mutagenicity, endocrine disruption or detoxification cascades.. We do know the particle size was small enough to reach the deepest lung where oxidative damage could be expected to take place, in addition to inflammation. We don't know anything about why the livers were abnormal though the liver is a major site of chemical detoxification.

The consensus statement points out that "…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." And, "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 mucocilliary "escalator" to the throat and swallowed", "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 inhalation testing was done by a "nose only" method that does not take into account any mouth breathing. And, we know that the upper respiratory system can be involved in inflammatory, oxidation and detoxification cascades.

Regardless of the weight or the number of particles, we have no experimental data on exactly how many of them reached what part of the lung or gut, and what lymph node, immune, cardio-pulmonary, inflammatory, oxidative or toxic effects they may have had, either acutely or, as the capsules degraded and the chemicals were slowly released. This is unfortunate, since there are methods to investigate these possibilities. We do know that 50% of the animals that inhaled LBAM-F had abnormal lungs, livers, or a combination of both. No hypothesis or reason has been offered for those abnormalities.

The consensus has downplayed the positive lymph node tests (LLNA), which evaluate early phase lymph system activation by measuring lymphocyte proliferation. The positivity of this test, rather than being questionable or of marginal importance, is more likely a landmark finding for how environmental chemicals effect and set the immune system on is future course. These findings may give profound insights into how and why there is a persistent increase in immune, neurological and inflammatory related illness in the population.

However the authors do discuss the possibility that the respiratory symptoms reported after the spray "were consistent with exposure to an irritant" and, "in general these conditions may be associated with exposure to a "sensitizer" or allergen." "… the positive LLNA result in Checkmate LBAM-F suggests a potential to cause this type of allergic reaction that cannot be dismissed." "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."

Evidence is accumulating that the immune, detoxification and anti-oxidant systems are intertwined, in the skin and elsewhere, and that lymph node activation and the development of dermal and respiratory sensitivity is not as simple as once thought.

We know that acute dermal local lymphocyte activation associated with respiratory sensitizers appears to produce Th2 activation with IL-4 production whereas dermal sensitizers produce Th1 activation with IFN-y and IL-4 production. Th1 and Th2 are different classes of T Helper Cell lymphocytes. But the LLNA with respiratory sensitizers appears to uniquely induce IL-4 so that IL-4 can be used as a differentiating factor.[4] There are various proposed expansions of the LLNA test and other tests to better quantify these, and other, issues.[5,6,7,8,9] In any case, the LBAM-F product could be a respiratory sensitizer. Unfortunately, this Th2, IL-4 related testing was not done, nor was evaluation of lung related lymph nodes.

One animal died after dermal exposure to LBAM-F, with lung, liver and spleen abnormalities. There was no hypothesis given as to why. Realizing that there are numerous lymph nodes in all areas of the body, including the lung area, it would be very interesting to see the results of lymph node testing on those areas, particularly the lung area. We do not know about the respiratory system lymph node reactivity, inflammatory and detoxification cascades or oxidation status. Again, we do not know why the livers were abnormal, nor do we know why there were abnormal spleens, the organ that is charged with filtering the blood and is rich with lymphocytes.

Studies have shown that lymphocyte activation does not happen independently, but is intricately involved with activation of detoxification cascades, with one modulating the other. Oxidative status regarding glutathione, and the detoxification system seem to be involved.

Skin sensitization requires low molecular weight compounds to penetrate the skin and bind to protein. The skin possesses a complex detoxification system, as does the lung, liver and other organs. A skin-like concoction of skin CYP detoxification isoenzymes

has been developed to test the metabolization of skin absorbed chemicals into allergenic adducts.[10] In other words, a seemingly non-allergic substance can be taken into the skin where detoxification enzymes can turn it into something more allergenic. These oxidation and metabolic products can then be tested for sensitization potential in the murine local lymph node assay (LLNA). This method has been used to determine the metabolic products of geraniol, a natural substance known to be metabolized into toxic allergenic adducts.[11] In that particular experiment, CYP2B6, CYP 1A1 and CYP3A5 showed high activities. We do not know all of the detoxification enzymes that are used by the various chemical constituents of the LBAM products, though we do know that BHT in the Checkmate LBAM-F uses CYP1B1 to form more toxic adducts.

Some of the possible detoxification adducts associated with the LBAM-F product, as well as genetic variances in detoxification enzymes and various inflammatory cascades have been discussed previously.[12]

Study has also shown that there is up regulation of the genes in the skin and lymph nodes in response to allergic contact dermatitis that have to do with plasma cells, mast cells and IFN-y such as IL-6, CCR-5, CCL-2, CCL-3, CXCL-1, CXCL-10, TIMP-1, OX-40, calgranulin b, ST2, ß-defensin, iNOS, STAT-1 MMP-3, MP-9, MMP-12 and MMP-13.[13]

How something that is an allergen at the skin and local lymph node area can also be a pulmonary sensitizer is unclear, but it does indeed appear to be the case. Respiratory sensitivity to Beryllium, for instance, has persisted despite institution of respiratory protection in the workplace. Dermal exposure with LLNA testing has found that the source of the respiratory sensitivity is dermal exposure.[14]

It has been shown that activation of one affected local lymphocyte area is transmitted to distant lymphocytes so that it is folly to think that local exposure cannot have more systemic consequences.[15]

Lastly, there appears to be a connection between skin cancer mutagens and carcinogens and their propensity to be skin sensitizers as well.[16] Again, the genotoxicity and carcinogenicity of the LBAM products have not been tested.

It is unclear how distant organs are potentially being affected by the test applications of the LBAM eradication products, but it is clear from the six-pack tests that organs are potentially being affected. This is a question of large importance given the huge, as well as expensive, problem of ongoing illness from a multitude of chronic diseases in this country.

Conclusion

This is a time of rapid expansion of our understanding of complex human systems. There is a shift from reductionist thinking to complex systems thinking. In part, this is driven by a growing understanding of genomics, epigenetics, toxicogenomics and complex inflammatory, immune, hormonal and signaling cascades.

There is no reason to think that the particular chemicals and capsules in the LBAM products are somehow immune to these principles..

There are many more questions to be answered about the proposed LBAM eradication chemicals. The testing done so far on the LBAM products falls far short of what is needed, yet does bring to light real possible dangers. To fully figure this out, we must embrace the newest of science.

What is clear is that there was an application of a pesticide product to a population who then presented with health complaints. That product, when inhaled in acute animal toxicity tests was associated with a 50% organ abnormality rate without reported explanation. That same product was associated with an animal death and abnormal organs upon dermal toxicity testing, without reported explanation. All products that were tested for dermal sensitization with the LLNA were positive. There was evidence of eye and dermal irritation. And, the most commonly used pesticide application at the present time, the twist tie, was not tested at all.

Despite the intended chronic exposure pattern planned for the LBAM products, the only testing we have is the acute six-pack testing.

Whatever we choose to expose ourselves and our children to, can and will have consequences far into the future. Whether we like it or not, that is the direction that our scientific knowledge is leading us. We cannot afford to throw precaution to the wind. Nor do we have the luxury of feigning ignorance. We know too much at this point, despite that knowledge being incomplete.

References

1. Review of Acute Toxicology Studies – Light Brown Apple Moth. California Department of Pesticide Regulation. Available at http://www.cdpr.ca.gov/docs/pressrls/lbam/lbam.htm

2. Deposition Study. Environmental monitoring results of the 2007 pheromone aerial applications for the light brown apple moth eradication project. California Department of Pesticide Regulation. Available at: http://www.cdfa.ca.gov/phpps/PDEP/lbam/reports.html

3. A Review of Acute Toxicity Studies Results on the Light Brown Apple Moth Pheromone Active Ingredient and Four LBAM Pheromone Products. Available at: http://www.oehha.ca.gov/pesticides/pdf/LBAMConsensus110308.pdf

4. Vandebriel RJ, De Jong WH, Spiekstra SW, Van Dijk M, Fluitman A, Garssen J, Van Loveren H. Assessment of preferential T-helper 1 or T-helper 2 induction by low molecular weight compounds using the local lymph node assay in conjunction with RT-PCR and ELISA for interferon-gamma and interleukin-4. *Toxicol Appl Pharmacol*, 2000 Jun 15;162(2):77-85

5. Gerberick GF, Vassallo JD, Bailey RE, Chaney JG, Morrall SW, Lepoittevin JP. Development of a peptide reactivity assay for screening contact allergens. *Toxicological Sciences*. 2004; 81(2):332-343; doi:10.1093/toxsci/kfh213

6. Fukuyama T, Ueda H, Hayashi K, Tajima Y, Shuto Y, Saito TR, Harada T, Kosaka T. Detection of low-level environmental chemical allergy by a long-term sensitization method. *Toxicology Letters*. 2008 July 30;180(1):1-8; doi:10.1016/j.toxlet.2008.05.001

7. Vanoirbeek JAJ, Tarkowski M, Vanhooren HM, de Vooght V, Nemery B, Hoet P. Validation of a mouse model of chemical-induced asthma using trimellitic anhydride, a respiratory sensitizer, and dinitrochlorobenzene, a dermal sensitizer. *Journal of Allergy and Clinical Immunology*. 2006 May; 117(5):1090-1097; doi:10.1016/j.jaci.2006.01.027

8. Fukuyama T, Ueda H, Hayashi K, Tajima Y, Shuto Y, Saito TR, Harada T, Kosaka T Use of long term dermal sensitization followed by intratracheal challenge method to identify low-dose chemical-induced respiratory allergic responses in mice. *Toxicology Letters* 2008 Oct 1;181(3);163-170; doi:10.1016/j.toxlet.2008.07.017

9. Isola D, Kimber I, Sarlo K, Lalko J, Sipes IG. Chemical respiratory allergy and occupational asthma: What are the key areas of uncertainty? *Journal of Applied Toxicology*. 2008; 28: 249-253; doi: 10.1002/jat.1336

10. Andresen Bergstrom M, Ott H, Carlsson A, Neis M, Zwadlo-Klarwasser G, Jonsson C, Merk HF, Karlberg AT, Baron J. A skin-like cytochrome P450 cocktail activates prohaptens to contact allergenic metabolites. *Journal of Investigative Dermatology* (2007) 127m 1145-1153m doi:10.1038/sj.jid.5700638; published online 23 Nov 2006

11. Hagvall L, Malte Baron, Borge A, Weidolf L, Merk H, Kerlberg AT. Cytochrome P450-mediated activation of the fragrance compound geraniol forms potent contact allergens. *Toxicology and Applied Pharmacology*. doi: 10.1016/j.taap.2008.08.014

12. Haiden A. The Light Brown Apple Moth Aerial Spray Campaign: The Health Hazards of Particles, Toxins, Inflammatory Cascades and Genomic Predisposition. 2008 May 15. Available at www.drhaiden.com/moth

13. Hartmann B, Staedtler F, Hartmann N, Meingassner J, First H. Gene expression profiling of skin and draining lymph nodes of rats affected with cutaneous contact hypersensitivity. *Inflammation Research*. 2006 Aug;55(2):322-334

14. Tinkle S, Antonini J, Roberts J, Salmen R, Depree K, Flint M. Cutaneous application of beryllium salts and oxide particles produces beryllium-specific peripheral sensitization in the C3H/HeOuJ Mice. *Beryllium Research Symposium: Basic Mechanisms and Human Health*, June 25-26, 2002, National Library of Medicine, Bethesda, MD

15. Borodin Y.I., Maiborodin I.V., Safina A.F., Strunkin D.N. The possibility of cytochrome P450 1A1/1A2 induction in cells of distant lymph nodes of rats after enteral treatment with benzo[a]pyrene. *Bulletin of Experimental Biology and Medicine*. 2005 Nov;140(5):564-567

16. Ashby J, Hilton J, Dearman RJ, Callander RD, Kimber I. Mechanistic relationship among mutagenicity, skin sensitization, and skin carcinogenicity. *Environmental Health Perspectives* 1993 April; 101(1):62-67